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(54) Title: ENHANCED EFFICACY OF LIPOSOMAL ANTISENSE DELIVERY

(57) Abstract

Pharmaceutical compositions for the treatment of pathologic conditions associated with the overexpression of ICAM-1 in a host. These pharmaceutical compositions comprise an effective amount of an ICAM-1 antisense molecule encapsulated in a lipid mixture which is typically a liposome or lipid particle. The lipid mixture will typically comprise at least two members selected from the group consisting of phospholipids, sterols and cationic lipids.

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ENHANCED EFFICACY OF LIPOSOMAL ANTISENSE DELIVERY

BACKGROUND OF THE INVENTION

Research aimed at developing therapeutic drugs based on the inhibition of protein expression by oligonucleotides has encountered a number of limitations. It has been demonstrated *in vitro* that a number of cell types exhibit low rates of intracellular delivery of antisense molecules. This is of great concern as antisense molecules are designed to bind to mRNA target sites located in the cytoplasm and nucleus of cells. *In vivo* studies have also indicated that many oligonucleotides are rapidly cleared from circulation; thus a limited time period exits in which the antisense molecule can interact with the target cells. Furthermore, a number of studies have demonstrated that oligonucleotides are quite toxic when administered *in vivo*.

Many of the limitations of free antisense, such as toxicity and nuclease digestion, can potentially be overcome by encapsulating antisense molecules in liposomes. When made as relatively small particles (approximately 100 nm in diameter), liposomes will passively accumulate at sites of inflammation by moving through the restructured vasculature. Numerous studies have determined that liposomes or lipid complexes have the ability to deliver oligonucleotides intracellularly through two mechanisms: cellular uptake of liposomes via endocytosis, and fusion of cationic liposomes with target cell membranes.

Comparisons of free and encapsulated oligonucleotides, indicate an enhanced stability for encapsulated oligos *in vitro* as the liposome prevents nuclease degradation. In contrast, comparison of free and encapsulated phosphorothioate oligonucleotides usually indicate no enhancement as the phosphorothioate oligos are themselves nuclease resistant. Thus the same amount of oligonucleotide can potentially be delivered to the cell whether it is a free phosphorothioate or an encapsulated phosphodiester oligonucleotide. Wang, *et al., Proc. Natl. Acad. Sci.* **92**:3318-3322 (1995). Despite such studies, no evidence has been presented which demonstrates that a conventional neutral liposome (*e.g.*, phosphatidylcholine/cholesterol) can disrupt the endosome to release its contents into the cytoplasm.

One approach to this problem has involved the use of cationic lipid vesicles. These cationic lipid vesicles form "complexes" with DNA, including plasmids and oligonucleotides. These complexes are not liposomes (*i.e.* an intact bilayer encapsulating an aqueous space) but are aggregates of lipid and DNA held together by electrostatic attraction between the cationic lipid and anionic nucleic acid. Recent literature indicates that complexes of cationic lipids in association with a fusogenic factor such as phosphatidylethanolamine or a fusion protein is required to achieve a significant antisense effect or gene transfection. Farhood, *et al.*, *Biochim. Biophys. Acta.* **1235**:289-295 (1995); Felgner, *et al.*, *J. Biol. Chem.* **269**:2550-2561 (1994); and Wrobel, *et al.*, *Biochim. Biophys. Acta.* **1235**:296-304 (1995).

Still other studies involving intravenous administration of antisense oligonucleotides in monkeys have indicated certain toxicities associated with this practice (see Galbraith, *et al.*, *Antisense Res. Dev.* **4**:201-206 (1994) and Cornish, *et al.*, *Antisense Res. Dev.* **3**:239-247 (1993)). The most likely cause for the observed toxicities is the activation of the complement system, releasing C3a and C5a, vasoactive cleavage products of C3 and C5, respectively. In this regard, both DNA and RNA have been shown to activate complement in various *in vitro* assays.

The complement system is a multi-protein cascade which serves as one of the first lines of defense against foreign particles which have entered the blood. The two basic mechanisms by which complement attacks foreign particles are by opsonization and cell lysis. Opsonization involves the covalent attachment of complement fragments, principally C3b and iC3b, to the surface of a particle or cell, which is then recognized by corresponding receptors present on macrophages. Cell lysis involves the assembly of a multiprotein complex, C5b-9, which perforates cell membranes and generates a pore.

Relatively little work has been published concerning the use of neutral phospholipid vesicles (liposomes) as delivery systems for antisense oligonucleotides (see, Juliano, *et al.*, *Antisense Research & Development* **2**:165-176 (1992)). Oligonucleotides are usually 15-25 bases in length and highly charged which means they cannot cross membranes by passive diffusion. The target site for antisense drugs is either the cytoplasm or nucleus and consequently the plasma membrane acts as an effective barrier. Despite this, some free antisense oligonucleotides have been shown active *in vitro* and *in vivo* (see, Juliano, *et al.*, *Antisense Research & Development* **2**:165-176 (1992); Bennett, *et al.*, *J. Immunol.* **152**:3530-3540 (1994); Bennett, *et al.*, *Adv. Pharmacol.* **28**:1-43

(1994); and Stepkowski, *et al., J. Immunol.* **153**:5336-5346 (1994)), furthermore there is some evidence that a receptor mediated uptake mechanism for oligonucleotides may be responsible. Even if a target cell or tissue does exhibit an ability to endocytose antisense molecules the process is not efficient and biological efficacy is normally only observed at very high concentrations. This is most likely because even after endocytosis the nucleotide must still cross the endosomal membrane to reach the cytoplasm before it is degraded by lysosomal enzymes which occurs at a very low frequency. In the case of anti ICAM-1 the free oligonucleotide does not appear to penetrate endothelial cells in culture but when complexed with cationic lipid/phosphatidylethanolamine (PE) complexes extensive uptake is observed. Such cationic lipid/PE complexes are known to disrupt intracellular endosomes and deliver nucleic acids into the cytoplasm. Once in the cytoplasm antisense oligonucleotides rapidly diffuse into the nucleus (Sixou, *et al., Nuc. Acids Res.* **22**:662-668 (1994)) and this is also observed for anti ICAM-1 delivered by aggregates or complexes of the oligonucleotides and lipids (see Bennett, *et al, Mol. Pharmacol.* **41**:1023-1033 (1992)). As noted above, these latter complexes or aggregates are not liposome formulations and do not use encapsulation methods for construction of the complex. Moreover, the complexes (which are not encapsulated oligonucleotide systems) have not been shown to disrupt endosomes and as a result, will only show intracellular delivery of the oligonucleotide in *in vitro* studies.

Extensive modifications to the structure of oligonucleotides have been made in an attempt to improve stability *in vivo* and to enhance membrane permeability. For example the phosphorothioates, noted above, in which an oxygen atom is replaced by a sulphur in the phosphate backbone, exhibit increased resistance to nucleases and are more stable *in vivo* than normal phosphodiester oligonucleotides (Juliano, *et al., Antisense Research & Development* **2**:165-176 (1992)). Another example are nucleic acid methylphosphonates, which are not only nuclease resistant but also hydrophobic analogues of phosphodiesters and therefore expected to be more membrane permeable (see, Hughes, *et al., J. Pharm. Sci.* **83**:597-600 (1994) and Tari, *et al., Blood* **84**:601-607 (1994)). Despite these developments, antisense oligonucleotide therapy is slow to develop mainly because it is difficult to get therapeutic drug levels into cells in target tissue and toxic side effects persist such as complement activation which restrict dosing.

Attractive targets for antisense therapy include the nucleic acids which encode intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1

(VCAM-1), and endothelial leukocyte adhesion molecule-1 (ELAM-1). ICAM-1, which is a 90-110 kDa membrane glycoprotein involved in the trafficking of leukocytes out of the vasculature and in antigen presentation to T cells (see Osborn, *Cell* **56**:907-910 (1990) and Springer, *Nature (Lond.)* **346**:425-443 (1990)). ICAM-1 is normally expressed at low levels on the surface of endothelial cells, keratinocytes, fibroblasts and leukocytes. Expression of ICAM-1 is inducible by a number of cytokines, including IL-1β, tumor necrosis factor-α and interferon-γ. Increased expression of ICAM-1 has been demonstrated in a variety of human diseases and has been shown to correlate with leukocyte infiltration in the diseased tissue.

What is needed in the art are new compositions and methods for the delivery of antisense molecules directed toward inhibiting the expression of cellular adhesion molecules. Such compositions should increase the serum stability of the antisense molecules and reduce toxic side effects such as complement activation. Surprisingly, the present invention provides such compositions and methods.

SUMMARY OF THE INVENTION

The present invention provides pharmaceutical compositions for the treatment of pathologic conditions associated with the overexpression of cellular adhesion molecules, such as ICAM-1 in a host. These pharmaceutical composition comprise an effective amount of an ICAM-1 antisense molecule encapsulated in a lipid mixture which is typically a liposome or lipid particle. The lipid mixture will typically comprise at least two members selected from the group consisting of phospholipids, sterols and cationic lipids.

In one group of embodiments, the antisense molecule is either a phosphorothioate molecule or a methyl phosphonate molecule, from about 15 to 50 nucleic acids, and is complementary to a portion of the 3'-untranslated region of ICAM-1.

For those embodiments in which the lipid mixture is a liposome composition, the liposome will preferably comprise phosphatidylcholine and cholesterol, more preferably egg phosphatidylcholine and cholesterol. For those embodiments in

which the lipid mixture is present as lipid particles, the particles will preferably comprise phospholipids and cationic lipids.

Additionally, the present invention provides methods for the treatment of pathologic conditions associated with the overexpression of ICAM-1 in a host. Such conditions include Alzheimer's disease, multiple sclerosis, uveitis, Herpes keratitis, renal allograft rejection, glomerulonephritis, liver allograft rejection, viral hepatitis, alcoholic hepatitis, cholangitis, cardiac allograft rejection, atherosclerotic plaques, rheumatoid arthritis, Grave's disease, Hashimoto's thyroiditis, psoriasis, scleroderma, graft v host disease, contact dermatitis, lichen planus, fixed drug eruption, mycosis fungoides, and alopecia areata.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 illustrates a spin column elution profile of encapsulated antisense. 50 μL of encapsulated antisense was applied to a 1 mL Biogel A15m, 200-400 mesh, spin column and separated. Lipid was determined by phosphate analysis (O), and oligonucleotide was detected by measuring A_{260}, after Bligh and Dyer extraction (●).

Figure 2 illustrates a purified liposomal antisense preparation. Liposome-encapsulated antisense was "purified" on DEAE-sepharose CL-6B columns. Removal of free antisense was assessed by size exclusion chromatography on 1 mL Biogel A15m column. Lipid was determined by phosphate analysis (O), and oligonucleotide was detected by measuring A_{260}, after Bligh and Dyer extraction (●).

Figure 3 shows a time course for leakage of encapsulated antisense. Leakage of encapsulated antisense was monitored at room temperature for 1 (O), 3 (■) and 5 (●) days.

Figure 4 shows the time course for leakage of encapsulated antisense at 4°C. Leakage of encapsulated antisense was monitored for 1 (O), 3 (□), and 5 (●) days, at 4°C.

Figure 5 illustrates complement activation by liposomes. Complement activation was investigated using an EPC/CH liposome preparation. Liposomes were 100 ± 30 nm. Lipid composition is expressed in molar ratios.

Figure 6 illustrates the ear swelling characteristics of ICR mice. Measurements refer to increases in ear thickness with respect to baseline measurements (prior to ear challenge). Values are given for two separate experiments. Error bars represent the standard deviation of measurements from 4 mice.

Figure 7 illustrates the ear swelling characteristics of BALB/c mice. Measurements refer to increases in ear thickness with respect to baseline measurements (prior to ear challenge). Error bars represent the standard deviation of measurements from 4 mice.

Figure 8 shows the liposome accumulation in the ears of ICR mice during various time intervals of inflammation. Liposomes were injected into mice at 0 hr, 24 hr, and 48 hr after initiation of ear inflammation. Liposomes were allowed to circulate for 24 hr at which time ears were recovered, digested, and analyzed for radiolabeled lipid. Error bars represent the standard deviation of measurements from 4 mice.

Figure 9 shows the liposome accumulation in the ears of BALB/c mice during various time intervals of inflammation. Liposomes were injected into mice at 0 hr, 24 hr, and 48 hr after initiation of ear inflammation. Liposomes were allowed to circulate for 24 hr at which time ears were recovered, digested, and analyzed for radiolabeled lipid. Error bars represent the standard deviation of measurements from 4 mice.

Figure 10 shows the MPO levels in the ears of ICR mice during inflammation. At various times after the initiation of inflammation, inflamed ears (■) and control ears (●) were recovered, homogenized, and assayed for MPO activity. Error bars represent the standard deviation of measurements from 4 mice.

Figure 11 shows cell infiltration into the inflamed ear of ICR mice during inflammation. Bone marrow cells and circulating leukocytes were labeled 24 hr prior to the onset of inflammation. At various times after the initiation of inflammation, ears were recovered, digested, and analyzed for radiolabeled cells by liquid scintillation counting. Error bars represent the standard deviation of measurements from 4 mice.

Figure 12 shows a typical inflammation experiment involving ICR mice. The following parameters were measured: ear swelling (▵); liposome accumulation in inflamed ( ) and non-inflamed (■) ears; and cell infiltration (O).

Figure 13 shows liposome accumulation in the ears of ICR mice during the first 24 hours of inflammation. DSPC:CH liposomes were injected into mice

immediately after initiation of ear inflammation. At various times mice were sacrificed and the ears were collected and analyzed (inflamed (○) and non-inflamed (□) ears). Error bars represent the standard deviation of measurements from 4 mice.

Figure 14 illustrates the circulation clearance rates of free [^{3}H]-antisense and liposome encapsulated [^{3}H]-antisense.

Figure 15 illustrates the tissue biodistribution of free [^{3}H]-antisense (Isis 2302).

Figure 16 illustrates the tissue biodistribution profiles for both the lipid and antisense portions of a lipsome encapsulated antisense formulation.

Figure 17 illustrates the ability of free antisense to inhibit ear inflammation.

Figure 18 illustrates the efficacy of free and encapsulated ICAM-1 antisense formulations in reducing ear inflammation in mice.

Figure 19 is a bar graph showing edema formation (based on ear weights) in mice treated with free and encapsulated antisense.

DETAILED DESCRIPTION OF THE INVENTION

Abbreviations and Definitions

Abbreviations used herein have the following meanings: i.v., intravenous; DC-Chol, 3β-(N-(N',N'-dimethylaminoethane)carbamoyl)cholesterol (see, Gao, *et al.*, *Biochem. Biophys. Res. Comm.* **179**:280-285 (1991)); DDAB, N,N-distearyl-N,N-dimethylammonium bromide; DMRIE, N-(1,2-dimyristyloxyprop-3-yl)-N,N-dimethyl-N-hydroxyethyl ammonium bromide; DODAC, N,N-dioleyl-N,N-dimethylammonium chloride (see commonly owned patent application USSN 08/316,399, incorporated herein by reference); DOGS, dioctadecylamidoglycyl carboxyspermine; DOPE, 1,2-*sn*-dioleoylphoshatidyethanolamine; DOSPA, N-(1-(2,3-dioleyloxy)propyl)-N-(2-(sperminecarboxamido)ethyl)-N,N-dimethylammonium trifluoroacetate; DOTAP, N-((2,3-dioleoyloxy)propyl)-N,N,N-trimethylammonium chloride; DOTMA, N-((2,3-dioleyloxy)propyl)-N,N,N-trimethylammonium chloride; EPC, egg phosphatidylcholine; RT, room temperature; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; HBS, HEPES buffered saline (150 mM NaCl and 20 mM

HEPES); PEG-Cer-C_{20}, 1-O-(2'-(ω-methoxypolyethyleneglycol)succinoyl)-2-N-arachidoyl-sphingosine; PEG-Cer-C_{14}, 1-O-(2'-(ω-methoxypolyethyleneglycol)succinoyl)-2-N-myristoyl-sphingosine; PBS, phosphate-buffered saline; EGTA, ethylenebis(oxyethylenenitrilo)-tetraacetic acid; OGP, n-octyl β-D-glycopyranoside (Sigma Chemical Co., St. Louis, MO); POPC, palmitoyl oleoyl phosphatidylcholine (Northern Lipids, Vancouver, BC); QELS, quasielastic light scattering; TBE, 89 mM Tris-borate with 2 mM EDTA; and EDTA, Ethylenediaminetetraacetic acid (Fisher Scientific, Fair Lawn, NJ); RES, reticuloendothelial system; SPDP, N-succinimidyl 3-(2-pyridyldithio) propionic acid; DTT, dithiothreitol; NEM, N-ethylmaleimide; Chol, cholesterol; DMPC, 1,2-*sn*-dimyristoylphosphatidylcholine; DSPC, 1,2-*sn*-distearoylphosphatidylcholine; EDTA, ethylenediaminetetraacetic acid; MLV, multilammelar vesicles; PEG-DSPE, poly(ethylene glycol)-modified distearoylphosphatidylethanolamine; LUV, large unilamellar vesicles; SUV, small unilamellar vesicles; PEG, poly(ethylene glycol); ^{3}H-CHE, ^{3}H-Cholesteryl hexadecyl ether.

The term "oligonucleotide" refers to a single or double-stranded polymer of deoxyribonucleotide or ribonucleotide bases read from the 5' to the 3' end. It includes both self-replicating plasmids, infectious polymers of DNA or RNA and non-functional DNA or RNA. When used to refer to oligonucleotides, the terms "phosphorothioate" and "methyl phosphonate" refer to those oligonucleotides in which a phosphodiester internucleotide linkage has been modified by replacing at least one of the non-bridged oxygens of the internucleotide linkage with sulfur or a methyl group, respectively. Preferably at least 10% of the internucleotide linkages are modified, more preferably at least 30% of the linkages are so modified. Most preferably, at least 50% of the linkages are modified.

The term "complementary" means that one nucleic acid hybridizes selectively to another nucleic acid. Selectivity of hybridization exists when hybridization (or base pairing) occurs that is more selective than total lack of specificity. Typically, selective hybridization will occur when there is at least about 55% paired bases over a stretch of at least 14-25 nucleotides, preferably at least about 65%, more preferably at least about 75%, and most preferably at least about 90%. See, M. Kanehisa *Nucleic Acids Res.* **12**:203 (1984), incorporated herein by reference.

The term "lipid" refers to any fatty acid derivative which is capable of forming a bilayer such that a hydrophobic portion of the lipid material orients toward the bilayer while a hydrophilic portion orients toward the aqueous phase. Hydrophilic characteristics derive from the presence of phosphato, carboxylic, sulfato, amino, sulfhydryl, nitro, and other like groups. Hydrophobicity could be conferred by the inclusion of groups that include, but are not limited to, long chain saturated and unsaturated aliphatic hydrocarbon groups and such groups substituted by one or more aromatic, cycloaliphatic or heterocyclic group(s). Preferred lipids are phosphoglycerides and sphingolipids, representative examples of which include phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, phosphatidic acid, palmitoyloleoyl phosphatidylcholine, lysophosphatidylcholine, lysophosphatidyl-ethanolamine, dipalmitoylphosphatidylcholine, dioleoylphosphatidylcholine, distearoyl-phosphatidylcholine or dilinoleoylphosphatidylcholine could be used. Other compounds lacking in phosphorus, such as sphingolipid and glycosphingolipid families are also within the group designated as lipid. Additionally, the amphipathic lipids described above may be mixed with other lipids including triglycerides and sterols.

The term "cationic lipid" refers to any of a number of lipid species which carry a net positive charge at physiological pH. Such lipids include, but are not limited to, DODAC, DOTMA, DDAB, DOTAP, DC-Chol and DMRIE. Additionally, a number of commercial preparations of cationic lipids are available which can be used in the present invention. These include, for example, LIPOFECTIN® (commercially available cationic liposomes comprising DOTMA and DOPE, from GIBCO/BRL, Grand Island, New York, USA); LIPOFECTAMINE® (commercially available cationic liposomes comprising DOSPA and DOPE, from GIBCO/BRL); and TRANSFECTAM® (commercially available cationic liposomes comprising DOGS from Promega Corp., Madison, Wisconsin, USA).

As used herein, the phrase "pathologic conditions associated with the overexpression of ICAM-1", is meant to include diseases of the central nervous system (*e.g.* Alzheimer's disease and multiple sclerosis), the eye (*e.g.* uveitis and Herpes keratitis), the kidney (*e.g.* renal allograft rejection and glomerulonephritis), the liver (*e.g.* liver allograft rejection, viral hepatitis, alcoholic hepatitis, cholangitis), the heart (*e.g.* cardiac allograft rejection and atherosclerotic plaques), the bone (*e.g.* rheumatoid arthritis), the thyroid (*e.g.* Grave's disease and Hashimoto's thyroiditis), and the skin

(*e.g.* psoriasis, scleroderma, graft v host disease, contact dermatitis, lichen planus, fixed drug eruption, mycosis fungoides, and alopecia areata).

As used herein, the term "host" refers to a human, rat, mouse, dog, cow, sheep, horse, cat and goat.

Description of the Embodiments

The present invention derives from the surprising discovery that antisense molecules which are encapsulated in a liposome or lipid particle composition can be delivered to a site of inflammation in response to overexpression of ICAM-1 and thereby reduce the associated inflammation. It was particularly surprising that liposome formulations which consist essentially of charge neutral lipids and a sterol (*e.g.*, cholesterol) would be effective for antisense delivery in view of the conventional wisdom that cationic liposome formulations or formulations having fusogenic lipids or proteins are necessary for cell or endosome fusion.

Accordingly, the present invention provides pharmaceutical compositions for the treatment of conditions associated with the overexpression of cellular adhesion molecules, preferably ICAM-1. These compositions comprise an antisense oligonucleotide encapsulated in a lipid mixture. The lipid mixture can be in either of two forms. The first is a conventional liposome, which is preferably charge neutral, consists essentially of neutral phospholipids and a sterol (*e.g.*, cholesterol) and which can be passively loaded with an antisense molecule. The second form is a lipid particle which comprises phospholipids, cationic lipids, sterols and combinations thereof.

Recently, others have described a cationic liposomal preparations of N-((2,3-dioleyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTMA) for the delivery of an ICAM-1 antisense molecule (see, Bennett, *et al.*, *Mol. Pharmacol.* **41**:1023-1033 (1992). The preparation used by Bennett, *et al.*, was a 1:1 mixture of DOTMA and dioleoyl phosphatidylethanolamine which formed 250 nm liposomes. These systems provided complexes of the antisense molecules which were not encapsulated. Moreover, these systems are useful only *in vitro* for intracellular delivery as they do not disrupt endosomes *in vivo*.

ANTISENSE OLIGONUCLEOTIDES

The antisense oligonucleotides which are useful in the present invention are those oligonucleotides which are complementary to a portion of a mammalian nucleic acid encoding cellular adhesion molecules such as ELAM-1 (human), VCAM-1 (human) and ICAM-1 (human and mouse) which are provided as Sequence I.Ds. No. 1, 3, 5 and 7, respectively):

ELAM-1 is a 115-kDa membrane glycoprotein which is a member of the selecting family of membrane glycoproteins (see, Bevilacqua, *et al.*, *Science*, 243:1160-1165 (1989)). The amino terminal region of ELAM-1 contains sequences with homologies to members of lectin-like proteins, followed by a domain similar to epidermal growth factor, followed by six tandem 60-amino acid repeats similar to those found in complement receptors 1 and 2. ELAM-1 is encoded by a 3.9-kb mRNA. The 3'-untranslated region of ELAM-1 mRNA contains sever sequence motifs ATTTA which are responsible for the rapid turnover of cellular mRNA consistent with the transient nature of ELAM-1 expression.

ELAM-1 exhibits a limited cellular distribution and has only been identified on vascular endothelial cells. Like ICAM-1, ELAM-1 is inducible by a number of cytokines including tumor necrosis factor, interleukin-1 and lymphotoxin and bacterial lipopolysaccharide. Unlike ICAM-1, ELAM-1 is not induced by gamma-interferon. The kinetics of ELAM-1 mRNA induction and disappearance in human umbilical endothelial cells precedes the appearance and disappearance of ELAM-1 on the cell surface.

VCAM-1 is a 110-kDa membrane glycoprotein encoded by a 3.2-kb mRNA. It appears to be encoded by a single-copy gene which can undergo alternative splicing to yield products with either six or seven immunoglobulin domains (see Osborn, *et al.*, *Cell* 59:1203-1211 (1989)). The receptor for VCAM-1 is proposed to be CD29 (VLA-4) as demonstrated by monoclonal antibodies which bind to CD29 and block the adherance of Ramos cells to VCAM-1. VCAM-1 is expressed primarily on vascular endothelial cells and is also regulated by treatment with cytokines (see, Rice, *et al.*, *Science* 246:1303-1306 (1989) and Rice, *et al.*, *J. Exp. Med.* 171:1369-1374 (1990)). Increased expression appears to be due to induction of the mRNA.

Human ICAM-1 is encoded by a 3.3-kb mRNA resulting in the synthesis of a 55,219 dalton protein. ICAM-1 is heavily glycosylated through N-linked

glycosylation sites. The mature protein has an apparent molecular mass of 90 kDa as determined by gel electrophoresis (see Staunton, *et al.*, *Cell* **52**:925-933 (1988)). ICAM-1 is a member of the immunoglobulin supergene family, containing 5 immunoglobulin-like domains at the amino terminus, followed by a transmembrane domain and a cytoplasmic domain. The primary binding site for LFA-1 and rhinovirus are found in the first immunoglobulin-like domain. However, the binding sites appear to be distinct.

ICAM-1 exhibits a broad tissue and cell distribution, and may be found on white blood cells, endothelial cells, fibroblast, keratinocytes and other epithelial cells. The expression of ICAM-1 can be regulated on vascular endothelial cells, fibroblasts, keratinocytes, astrocytes and several cell lines by treatment with bacterial lipopolysaccharide and cytokines such as interleukin-1, tumor necrosis factor, gamma-interferon, and lymphotoxin (see, *e.g.*, Frohman, *et al.*, *J. Neuroimmunol.* **23**:117-124 (1989)).

In preferred embodiments, the antisense oligonucleotide is complementary to the 3'-untranslated region of human ICAM-1 mRNA and contains from about 10 to about 50 nucleotides, more preferably about 15 to about 30 nucleotides. In particularly preferred embodiments, the antisense oligonucleotide is a phosphorothioate oligonucleotide or a methyl phosphonate oligonucleotide. Phosphorothioate oligonucleotides (PS-oligos) are those oligonucleotides in which one of the non-bridged oxygens of the internucleotide linkage has been replaced with sulfur. These PS-oligos are resistant to nuclease degradation, yet retain sequence-specific activity. Similarly, methyl phosphonate oligonucleotides (MeP-oligos) are those oligonucleotides in which one of the non-bridged oxygens of the internucleotide linkage has been replaced by a methyl group. These MeP-oligos have also proven to be more nuclease resistant than their natural phosphodiester linked derivatives. A number of antisense oligonucleotides which are directed toward inhibiting the production of ICAM-1, as well as VCAM-1 and ELAM-1, and which are useful in the present invention have been described in PCT applications: PCT/US91/05209; PCT/US94/09026; PCT/US94/12797 and PCT/US93/08101, the disclosures of each being incorporated herein by reference.

The antisense oligonucleotides used in the present invention may be synthesized in solid phase or in solution. Generally, solid phase synthesis is preferred. Detailed descriptions of the procedures for solid phase synthesis of oligonucleotides by

phosphite-triester, phosphotriester, and H-phosphonate chemistries are widely available. See, for example, Itakura, U.S. Pat. No. 4,401,796; Caruthers, *et al.*, U.S. Pat. Nos. 4,458,066 and 4,500,707; Beaucage, *et al.*, *Tetrahedron Lett.*, **22**:1859-1862 (1981); Matteucci, *et al.*, *J. Am. Chem. Soc.*, **103**:3185-3191 (1981); Caruthers, *et al.*, *Genetic Engineering*, 4:1-17 (1982); Jones, chapter 2, Atkinson, *et al.*, chapter 3, and Sproat, *et al.*, chapter 4, in *Oligonucleotide Synthesis: A Practical Approach*, Gait (ed.), IRL Press, Washington D.C. (1984); Froehler, *et al.*, *Tetrahedron Lett.*, 27:469-472 (1986); Froehler, *et al.*, *Nucleic Acids Res.*, 14:5399-5407 (1986); Sinha, *et al. Tetrahedron Lett.*, 24:5843-5846 (1983); and Sinha, *et al.*, *Nucl. Acids Res.*, 12:4539-4557 (1984) which are incorporated herein by reference.

Generally, the timing of delivery and concentration of monomeric nucleotides utilized in a coupling cycle will not differ from the protocols typical for commercial phosphoramidites used in commercial DNA synthesizers. In these cases, one may merely add the solution containing the monomers to a receptacle on a port provided for an extra phosphoramidite on a commercial synthesizer (*e.g.*, model 380B, Applied Biosystems, Foster City, California, U.S.A.). However, where the coupling efficiency of a particular monomer is substantially lower than the other phorphoramidites, it may be necessary to alter the timing of delivery or the concentration of the reagent in order to optimize the synthesis. Means of optimizing oligonucleotide synthesis protocols to correct for low coupling efficiencies are well known to those of skill in the art. Generally one merely increases the concentration of the reagent or the amount of the reagent delivered to achieve a higher coupling efficiency. Methods of determining coupling efficiency are also well known. For example, where the 5'-hydroxyl protecting group is dimethoxytrityl (DMT), coupling efficiency may be determined by measuring the DMT cation concentration during the acidic removal of the DMT group. DMT cation concentration is usually determined by spectrophotometrically monitoring the acid wash. The acid/DMT solution is a bright orange color. Alternatively, since capping prevents further extension of an oligonucleotide where coupling has failed, coupling efficiency may be estimated by comparing the ratio of truncated to full length oligonucleotides utilizing, for example, capillary electrophoresis or HPLC.

Solid phase oligonucleotide synthesis may be performed using a number of solid supports. A suitable support is one which provides a functional group for the attachment of a protected monomer which will become the 3' terminal base in the

synthesized oligonucleotide. The support must be inert to the reagents utilized in the particular synthesis chemistry. Suitable supports are well known to those of skill in the art. Solid support materials include, but are not limited to polacryloylmorpholide, silica, controlled pore glass (CPG), polystyrene, polystyrene/latex, and carboxyl modified teflon. Preferred supports are amino-functionalized controlled pore glass and carboxyl-functionalized teflon.

Solid phase oligonucleotide synthesis requires, as a starting point, a fully protected monomer (*e.g.*, a protected nucleoside) coupled to the solid support. This coupling is typically through the 3'-hydroxyl. Typically, a linker group is covalently bound to the 3'-hydroxyl on one end and covalently bound to the solid support on the other end. The first synthesis cycle then couples a nucleotide monomer, via its 3'-phosphate, to the 5'-hydroxyl of the bound nucleoside through a condensation reaction that forms a 3'-5' phosphodiester linkage. Subsequent synthesis cycles add nucleotide monomers to the 5'-hydroxyl of the last bound nucleotide. In this manner an oligonucleotide is synthesized in a 3' to 5' direction producing a "growing" oligonucleotide with its 3' terminus attached to the solid support.

Numerous means of linking nucleoside monomers to a solid support are known to those of skill in the art, although monomers covalently linked through a succinate or hemisuccinate to controlled pore glass are generally preferred. Conventional protected nucleosides coupled through a hemisuccinate to controlled pore glass are commercially available from a number of sources (*e.g.*, Glen Research, Sterling, Vermont, U.S.A.; Applied Biosystems, Foster City, California, U.S.A.; and Pharmacia LKB, Piscataway, New Jersey, U.S.A.).

Once the full length oligonucleotide is synthesized, the oligonucleotide is deprotected and cleaved from the solid support prior to use. Cleavage and deprotection may occur simultaneously or sequentially in any order. The two procedures may be interspersed so that some protecting groups are removed from the oligonucleotide before it is cleaved off the solid support and other groups are deprotected from the cleaved oligonucleotide in solution. The sequence of events depends on the particular blocking groups present, the particular linkage to a solid support, and the preferences of the individuals performing the synthesis. Where deprotection precedes cleavage, the protecting groups may be washed away from the oligonucleotide which remains bound on the solid support. Conversely, where deprotection follows cleavage, the removed

protecting groups will remain in solution with the oligonucleotide. Often the oligonucleotide will require isolation from these protecting groups prior to use.

In a preferred embodiment, and most commercial DNA syntheses, the protecting group on the 5'-hydroxyl is removed at the last stage of synthesis. The oligonucleotide is then cleaved off the solid support, and the remaining deprotection occurs in solution. Removal of the 5'-hydroxyl protecting group typically requires treatment with the same reagent utilized throughout the synthesis to remove the terminal 5'-hydroxyl protecting groups prior to coupling the next nucleotide monomer. Where the 5'- hydroxyl protecting group is a dimethoxytrityl group, deprotection can be accomplished by treatment with acetic acid, dichloroacetic acid or trichloroacetic acid.

Where the oligonucleotide is a ribonucleotide and the 2'-hydroxyl group is blocked with a tert-butyldimethylsilyl (TBDMS) moiety, the latter group may be removed using tetrabutylammonium fluoride in tetrahydrofuran at the end of synthesis. See Wu, *et al.*, *J. Org. Chem.* 55:4717-4724 (1990). Phenoxyacetyl protecting groups can be removed with anhydrous ammonia in alcohol (under these conditions the TBDMS groups are stable and the oligonucleotide is not cleaved). The benzoyl protecting group of cytidine is also removed with anhydrous ammonia in alcohol.

Cleaved and fully deprotected oligonucleotides may be used directly (after lyophilization or evaporation to remove the deprotection reagent) or they may be purified prior to use. Purification of synthetic oligonucleotides is generally desired to isolate the full length oligonucleotide from the protecting groups that were removed in the deprotection step and, more importantly, from the truncated oligonucleotides that were formed when oligonucleotides that failed to couple with the next nucleotide monomer were capped during synthesis.

Oligonucleotide purification techniques are well known to those of skill in the art. Methods include, but are not limited to, thin layer chromatography (TLC) on silica plates, gel electrophoresis, size fractionation (*e.g.*, using a Sephadex column), reverse phase high performance liquid chromatography (HPLC) and anion exchange chromatography (*e.g.*, using the mono-Q column, Pharmacia-LKB, Piscataway, New Jersey, U.S.A.). For a discussion of oligonucleotide purification see McLaughlin, *et al.*, chapter 5, and Wu, *et al.*, chapter 6 in *Oligonucleotide Synthesis: A Practical Approach*, Gait (ed.), IRL Press, Washington, D.C., (1984).

To test the therapeutic ability of liposomes as delivery vehicles for antisense molecules to ICAM-1, ISIS Pharmaceuticals donated their antisense molecules ISIS 2302 (human, Sequence I.D. No. 9) and 3082 (murine, Sequence I.D. No. 10) towards the project. These oligonucleotides are phosphorothioate molecules in which each phosphodiester linkage is a phosphorothioate diester linkage. Additionally, each oligonucleotide consists of 20 nucleotides specifically designed to bind to a sequence in the 3' untranslated region of ICAM-1 mRNA. Currently it is theorized that this antisense molecule acts posttranscriptionally to inhibit the expression of ICAM-1 via 2 possible mechanisms: inhibiting the formation of a stabilizing stem-loop structure necessary for mRNA stability, and by increasing the degradation of the target mRNA via increased RNase-H degradation.

Preparation of pharmaceutical compositions containing the antisense oligonucleotides will typically involve either encapsulating the oligonucleotide in a liposome or forming a lipid particle in which the oligonucleotide is coated with a lipid mixture.

LIPOSOME-ENCAPSULATED ANTISENSE OLIGONUCLEOTIDES

The liposomes which are used in the present invention are formed from standard vesicle-forming lipids, which generally include neutral and negatively charged phospholipids, preferably neutral phospholipids, and a sterol, such as cholesterol. The selection of lipids is generally guided by consideration of, *e.g.*, liposome size and stability of the liposomes in the bloodstream.

Typically, the major lipid component in the liposomes is phosphatidylcholine. Phosphatidylcholines having a variety of acyl chain groups of varying chain length and degree of saturation are available or may be isolated or synthesized by well-known techniques. In general, less saturated phosphatidylcholines are more easily sized, particularly when the liposomes must be sized below about 0.3 microns, for purposes of filter sterilization. Phosphatidylcholines containing saturated fatty acids with carbon chain lengths in the range of C_{14} to C_{22} are preferred. Phosphatidylcholines with mono or diunsaturated fatty acids and mixtures of saturated and unsaturated fatty acids may also be used. Other suitable lipids include phosphonolipids in which the fatty acids are linked to glycerol via ether linkages rather than ester linkages. Liposomes useful in the present invention may also be composed of

sphingomyelin or phospholipids with head groups other than choline, such as ethanolamine, serine, glycerol and inositol. Preferred liposomes will include a sterol, preferably cholesterol, at molar ratios of from 0.1 to 1.0 (cholesterol:phospholipid). Most preferred liposome compositions are egg phosphatidylcholine/cholesterol, distearoylphosphatidylcholine/cholesterol, dipalmitoylphosphatidylcholine/cholesterol, and sphingomyelin/cholesterol. Methods used in sizing and filter-sterilizing liposomes are discussed below.

The liposomes can be prepared by any of the techniques now known or subsequently developed for preparing liposomes. For example, the liposomes can be formed by the conventional technique for preparing multilamellar lipid vesicles (MLVs), that is, by depositing one or more selected lipids on the inside walls of a suitable vessel by dissolving the lipids in chloroform and then evaporating the chloroform, and by then adding the aqueous solution which is to be encapsulated to the vessel, allowing the aqueous solution to hydrate the lipid, and swirling or vortexing the resulting lipid suspension. This process engenders a mixture including the desired liposomes.

Alternatively, techniques used for producing large unilamellar lipid vesicles (LUVs), such as reverse-phase evaporation, infusion procedures, and detergent dilution, can be used to produce the liposomes. A review of these and other methods for producing lipid vesicles can be found in the text Liposome Technology, Volume I, Gregory Gregoriadis Ed., CRC Press, Boca Raton, Florida, (1984), which is incorporated herein by reference. For example, the lipid-containing particles can be in the form of steroidal lipid vesicles, stable plurilamellar lipid vesicles (SPLVs), monophasic vesicles (MPVs), or lipid matrix carriers (LMCs) of the types disclosed in Lenk, *et al.* U.S. Patent No. 4,522,803, and Fountain, *et al.* U.S. Patent Nos. 4,588,578 and 4,610,868, the disclosures of which are incorporated herein by reference. A particularly preferred method for preparing LUVs is described in U.S. Patent No. 5,008,050.

Additionally, in the case of MLVs, if desired, the liposomes can be subjected to multiple (five or more) freeze-thaw cycles to enhance their trapped volumes and trapping efficiencies and to provide a more uniform interlamellar distribution of solute (Mayer, *et al., J. Biol. Chem.* **260**:802-808 (1985)).

Following liposome preparation, the liposomes may be sized to achieve a desired size range and relatively narrow distribution of liposome sizes. In preferred

embodiments, the liposomes will have diameters of from about 50 to about 150 nm, more preferably from about 75 to about 125 nm.

Several techniques are available for sizing liposomes to a desired size. One sizing method is described in U.S. Pat. No. 4,737,323, incorporated herein by reference. Sonicating a liposome suspension either by bath or probe sonication produces a progressive size reduction down to small unilamellar vesicles (SUVs) less than about 0.05 microns in size. Homogenization is another method which relies on shearing energy to fragment large liposomes into smaller ones. In a typical homogenization procedure, multilamellar vesicles are recirculated through a standard emulsion homogenizer until selected liposome sizes, typically between about 0.1 and 0.5 microns, are observed. In both methods, the particle size distribution can be monitored by conventional laser-beam particle size determination.

Extrusion of liposome through a small-pore polycarbonate membrane or an asymmetric ceramic membrane is also an effective method for reducing liposome sizes to a relatively well-defined size distribution (see, U.S. Patent No. 5,008,050 and Hope, *et al.*, in: *Liposome Technology, vol. 1, 2d ed.* (G. Gregoriadis, Ed.) CRC Press, pp. 123-139 (1992), the disclosures of which are incorporated herein by reference). Typically, the suspension is cycled through the membrane one or more times until the desired liposome size distribution is achieved. The liposomes may be extruded through successively smaller-pore membranes, to achieve a gradual reduction in liposome size. For use in the present inventions, liposomes having a size of from about 0.05 microns to about 0.15 microns are preferred. Other useful sizing methods such as sonication, solvent vaporization or reverse phase evaporation are known to those of skill in the art.

Liposomes prepared for use in the methods and pharmaceutical compositions of the present invention may be dehydrated for longer storage. The liposomes are preferably dehydrated under reduced pressure using standard freeze-drying equipment or equivalent apparatus. The lipid vesicles and their surrounding medium can also be frozen in liquid nitrogen before being dehydrated or not, and placed under reduced pressure. Dehydration without prior freezing takes longer than dehydration with prior freezing, but the overall process is gentler without the freezing step, and thus there is subsequently less damage to the lipid vesicles and a smaller loss of the internal contents. Dehydration without prior freezing at room temperature and at a reduced pressure provided by a vacuum pump capable of producing a pressure of about 1 mm Hg

typically takes between approximately 24 and 36 hours, while dehydration with prior freezing under the same conditions generally takes between approximately 12 and 24 hours.

To ensure that the liposomes will survive the dehydration process without losing a substantial portion of their internal contents, it is important that one or more protective sugars be available to interact with the lipid vesicle membranes and keep them intact as the water in the system is removed. A variety of sugars can be used, including such sugars as trehalose, maltose, sucrose, glucose, lactose, and dextran. In general, disaccharide sugars have been found to work better than monosaccharide sugars, with the disaccharide sugars trehalose and sucrose being most effective. Other more complicated sugars can also be used. For example, aminoglycosides, including streptomycin and dihydrostreptomycin, have been found to protect lipid vesicles during dehydration.

Typically, one or more sugars are included as part of either the internal or external media of the lipid vesicles. Most preferably, the sugars are included in both the internal and external media so that they can interact with both the inside and outside surfaces of the liposomes' membranes. Inclusion in the internal medium is accomplished by adding the sugar or sugars to the buffer which becomes encapsulated in the lipid vesicles during the lipid vesicle formation process. Since in most cases this buffer also forms the bathing medium for the finished lipid vesicles, inclusion of the sugars in the buffer also makes them part of the external medium.

The amount of sugar to be used depends on the type of sugar used and the characteristics of the lipid vesicles to be protected. See, U.S. Patent No. 4,880,635 and Harrigan, *et al.*, *Chem. Phys. Lipids* **52**:139-149 (1990), the disclosures of which are incorporated herein by reference. Persons skilled in the art can readily test various sugar types and concentrations to determine which combination works best for a particular lipid vesicle preparation. In general, sugar concentrations on the order of 100 mM and above have been found necessary to achieve the highest levels of protection. In terms of moles of membrane phospholipid, millimolar levels on the order of 100 mM correspond to approximately 5 moles of sugar per mole of phospholipid.

In the case of dehydration without prior freezing, if the lipid vesicles being dehydrated are of the type which have multiple lipid layers and if the dehydration is carried to an end point where between about 2% and about 5% of the original water in

the preparation is left in the preparation, the use of one or more protective sugars may be omitted.

Once the lipid vesicles have been dehydrated, they can be stored for extended periods of time until they are to be used. The appropriate temperature for storage will depend on the make up of the lipid vesicles and the temperature sensitivity of whatever materials have been encapsulated in the lipid vesicles. For example, as is known in the art, various oligonucleotides are heat labile, and thus dehydrated lipid vesicles containing such oligonucleotides should be stored under refrigerated conditions so that the potency of the agent is not lost. Also, for such agents, the dehydration process is preferably carried out at reduced temperatures, rather than at room temperature.

Methods of loading antisense oligonucleotides into liposomes will typically be carried out using an encapsulation technique in which the antisense oligonucleotide is placed into a buffer and added to a dried film of only lipid components. In this manner, the oligonucleotide will become encapsulated in the aqueous interior of the liposome. The buffer which is used in the formation of the liposomes can be any biologically compatible buffer solution of, for example, isotonic saline, phosphate buffered saline, or other low ionic strength buffers. Generally, the antisense oligonucleotide will be present in an amount of from about 0.01 ng/mL to about 200 mg/mL. The resulting liposomes with the antisense oligonucleotide incorporated in the aqueous interior or in the membrane are then optionally sized as described above.

Alternatively, the antisense oligonucleotide can be formulated in lipid particles such as those described in co-pending U.S. Ser. Nos. 08/484,282 and 08/485,458 each being filed on June 7, 1995 and incorporated herein by reference.

ANTISENSE LIPID PARTICLES

Antisense lipid particles can be prepared by combining an antisense oligonucleotide with cationic lipids in a detergent solution to provide a coated antisense-lipid complex. The complex is then contacted with phospholipids to provide a solution of detergent, an antisense-lipid complex and phospholipids, and the detergent is then removed to provide a solution of serum-stable antisense-lipid particles, in which the

antisense oligonucleotide is encapsulated in a lipid bilayer. The particles, thus formed, have a size of about 50-150 nm.

Alternatively, serum-stable antisense-lipid particles can be formed by preparing a mixture of cationic lipids and phospholipids in an organic solvent; contacting an aqueous solution of the antisense oligonucleotide with the mixture of cationic and phospholipids to provide a clear single phase; and removing the organic solvent to provide a suspension of antisense-lipid particles, in which the antisense oligonucleotide is encapsulated in a lipid bilayer, and the particles are stable in serum and have a size of about 50-150 nm.

In this aspect of the invention, cationic lipids which are useful will include, for example, DODAC, DOTMA, DDAB, DOTAP, DC-Chol, DORI and DMRIE. These lipids and related analogs, which are also useful in the present invention, have been described in co-pending USSN 08/316,399; U.S. Patent Nos. 5,208,036, 5,264,618, 5,279,833, 5,283,185, and 5,334,761, the disclosures of which are incorporated herein by reference. Additionally, a number of commercial preparations of cationic lipids are available and can be used in the present invention. These include, for example, LIPOFECTIN® (commercially available cationic liposomes comprising DOTMA and DOPE, from GIBCO/BRL, Grand Island, New York, USA); LIPOFECTAMINE® (commercially available cationic liposomes comprising DOSPA and DOPE, from GIBCO/BRL); and TRANSFECTAM® (commercially available cationic liposomes comprising DOGS from Promega Corp., Madison, Wisconsin, USA).

An initial solution of coated antisense-lipid complexes is formed by combining the antisense oligonucleotides with the cationic lipids in a detergent solution. The detergent solution is preferably an aqueous solution of a neutral detergent having a critical micelle concentration of 15-300 mM, more preferably 20-50 mM. Examples of suitable detergents include, for example, N,N'-((octanoylimino)-bis-(trimethylene))-bis-(D-gluconamide) (BIGCHAP); BRIJ 35; Deoxy-BIGCHAP; dodecylpoly(ethylene glycol) ether; Tween 20; Tween 40; Tween 60; Tween 80; Tween 85; Mega 8; Mega 9; Zwittergent® 3-08; Zwittergent® 3-10; Triton X-405; hexyl-, heptyl-, octyl- and nonyl-β-D-glucopyranoside; and heptylthioglucopyranoside; with octyl β-D-glucopyranoside being the most preferred. The concentration of detergent in the detergent solution is typically about 100 mM to about 2 M, preferably from about 200 mM to about 1.5 M.

The antisense oligos, cationic lipids and phospholipids which are useful in this group of embodiments are as described for the detergent dialysis preparative methods described above.

The selection of an organic solvent will typically involve consideration of solvent polarity and the ease with which the solvent can be removed at the later stages of particle formation. The organic solvent, which is also used as a solubilizing agent, is in an amount sufficient to provide a clear single phase mixture of antisense oligonucleotide and lipids. Suitable solvents include chloroform, dichloromethane, diethylether, cyclohexane, cyclopentane, benzene, toluene, methanol, or other aliphatic alcohols such as propanol, isopropanol, butanol, tert-butanol, iso-butanol, pentanol and hexanol. Combinations of two or more solvents may also be used in the present invention.

Contacting the antisense oligonucleotide with the organic solution of cationic and phospholipids is accomplished by mixing together a first solution of antisense oligonucleotide, which is typically an aqueous solution and a second organic solution of the lipids. One of skill in the art will understand that this mixing can take place by any number of methods, for example by mechanical means such as by using vortex mixers.

After the antisense oligonucleotide has been contacted with the organic solution of lipids, the organic solvent is removed, thus forming an aqueous suspension of serum-stable antisense-lipid particles. The methods used to remove the organic solvent will typically involve evaporation at reduced pressures or blowing a stream of inert gas (*e.g.*, nitrogen or argon) across the mixture.

The serum-stable antisense-lipid particles thus formed will typically be sized from about 50 nm to 150 nm. To achieve further size reduction or homogeneity of size in the particles, sizing can be conducted as described above.

ADMINISTRATION OF THE ANTISENSE FORMULATIONS

The present invention further provides methods for the treatment of pathologic conditions associated with the overexpression of ICAM-1 in a host. In these methods a pharmaceutical composition as described above is administered to the host. In preferred embodiments, the host is a mammal, more preferably a mouse, rat, human, horse, dog, cat, cow or pig. Still more preferably, the host is human. Preferred

antisense oligonucleotides, lipid mixtures and lipids are as described above for the compounds of the present invention.

The antisense oligonucleotide liposomes and lipid particles described above can be administered in any suitable manner, preferably with pharmaceutically acceptable carriers. One skilled in the art will appreciate that suitable methods of administering such compositions in the context of the present invention to an animal are available, and, although more than one route can be used to administer a particular composition, a particular route can provide a more immediate and more effective reaction than another route. Pharmaceutically acceptable carriers are also well-known to those who are skilled in the art. The choice of carrier will be determined in part by the particular composition, as well as by the particular method used to administer the composition. Accordingly, there is a wide variety of suitable formulations of the pharmaceutical composition of the present invention.

Formulations suitable for oral administration can consist of (a) liquid solutions, such as an effective amount of the antisense oligonucleotide (in a liposome or lipid particle) dissolved in diluents, such as water, saline or PEG 400; (b) suspensions in an appropriate liquid; and (c) suitable emulsions.

Formulations suitable for parenteral administration, such as, for example, by intravenous, intramuscular, intradermal, intraperitoneal, and subcutaneous routes, include aqueous and non-aqueous, isotonic sterile injection solutions, which can contain antioxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient, and aqueous and non-aqueous sterile suspensions that can include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives. The formulations can be presented in unit-dose or multi-dose sealed containers, such as ampules and vials, and can be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example, water, for injections, immediately prior to use. Extemporaneous injection solutions and suspensions can be prepared from sterile powders, granules, and tablets of the kind previously described.

The dose administered to an animal, particularly a human, in the context of the present invention should be sufficient to effect a therapeutic response in the animal over a reasonable time frame. The dose will be determined by the strength of the particular compound employed and the condition of the animal, as well as the body

weight or surface area of the animal to be treated. The size of the dose also will be determined by the existence, nature, and extent of any adverse side-effects that might accompany the administration of a particular compound in a particular animal.

As noted, the antisense oligonucleotides (in liposome or lipid particle formulations) can be administered, for example, by intravenous infusion, orally, topically, intraperitoneally, intravesically or intrathecally.

For oral administration, the compositions of the present invention can be administered at the rate up to 3000 mg/m^2 body surface area, which approximates 6 grams/day in the average patient. A preferred rate is from 1 to 300 mg/m^2 body surface area. This can be accomplished via single or divided doses. For intravenous administration, such compounds can be administered at the rate of up to about 2500 mg/m^2/d, preferably from about 0.1 to about 200 mg/m^2/d. For intravesicle administration, such compounds can be administered at the rate of up to about 2500 mg/m^2/d, preferably from about 0.1 to about 200 mg/m^2/d. For topical administration, the rate can be up to about 2500 mg/m^2/d, preferably from about 0.1 to about 200 mg/m^2/d. The dose for inhalation/aerosol administration can be up to about 2500 mg/m^2/d, preferably from about 0.1 to about 200 mg/m^2/d. Direct intraperitoneal administration can be performed using up to about 3000 mg/m^2/d, preferably from about 0.1 to about 100 mg/m^2/d. The dose for reservoir administration to the brain or spinal fluid can be up to about 2000 mg/m^2/d, preferably from about 0.1 to about 100 mg/m^2/d. For slow release intraperitoneal or subcutaneous administration, the dose can be from about 0.1 to about 5000 mg/day in a bolus, preferably from about 1.0 to about 200 mg/day. For intrathecal administration, the dose can be up to about 2000 mg/m^2/d, preferably from about 0.1 to about 100 mg/m^2/d.

The following examples are offered solely for the purposes of illustration, and are intended neither to limit nor to define the invention.

EXAMPLES

Materials

1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC) and egg phosphatidylcholine (EPC) was purchased from Avanti Polar Lipids (Alabaster, Alabama,

USA). Cholesterol, N-(2-hydroxyethyl) piperazine-N'-(2-ethanesulphonic acid) (HEPES), SEPHAROSE® CL-4B, SEPHADEX® G-25, SEPHADEX® G-50 and dithiothreitol (DTT) were obtained from Sigma Chemical Co. (St. Louis, Missouri, USA). ^{3}H-Cholesteryl hexadecyl ether (^{3}H-CHE) was obtained from Amersham (Oakville, Ontario, Canada). Female ICR and Balb/c mice were obtained from Harley-Sprague Davis.

Buffers

5X Veronal Buffered Saline (VBS). Mix 85.0 g of NaCl, 3.75 g of sodium barbitone, and 5.75 g of barbitone in 1.6 L of distilled water. Heat the mixture to dissolve the barbitone. Bring the volume up to 2.0 L with distilled water. The pH should be 7.4 - 7.6. Store at 4°C.

GVB^{2-}. Dissolve 0.5 g of gelatin in 100 mL of distilled water and heat to dissolve. Add 100 mL of 5X VBS and make the total volume to 500 mL with distilled water. Store at 4°C. Make this buffer every few days.

D5W^{2-}. Dissolve 25 g of D-glucose in 500 mL of distilled water. Make this solution every few days. Store at 4°C.

DGVB^{2+}. Mix 250 mL of D5W with 250 mL of GVB^{2-}. Add 75 μL of 1.0 M $CaCl_2$ and 100 μL of 2.5 M $MgCl_2$. Store at 4°C. Make this buffer every few days.

EDTA•GVB^{2-}. Mix 1.489 g EDTA, 0.5 g gelatin, 100 mL of 5X VBS, and 200 mL of distilled water. Bring the pH up to 7.4 with 5M NaOH (this will also help to dissolve the EDTA). Make the volume up to 500 mL with distilled water. Store at 4°C. Make this buffer every few days.

EXAMPLE 1

This example illustrates the passive encapsulation of antisense oligonucleotides in liposomes. Additionally, this example illustrates that liposomes and unencapsulated DNA can be separated by their molecular weight difference using size exclusion chromatography.

1.1 Encapsulation of antisense

Egg phosphatidylcholine (EPC) (1.78 g) and cholesterol (CH) (0.72 g) were weighed out in a laminar flow hood and combined in chloroform in a 150 mL round bottomed flask. Chloroform was removed by roto-evaporation for 3 hours.

The antisense molecule ISIS 2302 (1.5 g) was dissolved in 10 mL of sterile phosphate buffered saline (PBS) and added to the lipid film composed of 2.5 g of EPC:CH (55:45; mol:mol). The resulting multilamellar vesicles (MLV) were transferred to three 5 mL cryovials and subjected to five cycles of freezing in liquid nitrogen and thawing at 40°C. The freeze-thawed MLV were combined, transferred into a sterile 100 mL capacity extruder (Lipex Biomembranes, Vancouver) and extruded ten times through one 0.1 μm filter.

Similar conditions were followed for smaller liposomal antisense preparations, except that 200 mg of oligonucleotide was rehydrated in 250 mg (total) of EPC:CH lipid.

1.2 DEAE-Sepharose Chromatography

Non-encapsulated (free) antisense molecule was separated from the entrapped molecule by anion exchange chromatography using DEAE-Sepharose CL-6B. In order to ensure reasonable sterility of the column material, the columns were washed with sterile PBS followed by elution of 300 mL of 100 nm vesicles composed of EPC (24 mg/mL in PBS). Columns consisted of 6 mL syringes with a packed volume of 5 mL. A 400 mL aliquot of the antisense-liposome suspension was eluted on each column and the fractions containing lipid were collected and combined. Each column was used twice and then stored at 4°C for future recovery of the free antisense molecule.

The total volume of the column eluant was approximately 110 mL; therefore, it was necessary to concentrate the liposomal antisense. The liposomal antisense was placed in dialysis tubing (MW cutoff, 14000) and concentrated to approximately 28 mL using Aquacide. Results of this purification are shown in Figure 2.

1.3 Measurement of Trapping Efficiency

Trapping efficiency was determined by size exclusion chromatography using a 1 mL Biogel A15m (fine) spin column as described previously (Chonn *et al.*,

1991). Briefly, a 50 μL aliquot of the liposomal antisense was diluted with 70 μL of PBS, and 50 μL was eluted on each of two duplicate columns. Each fraction consisted of the volume eluted (~40 μL) during centrifugation at 1000 rpm for 1 min. The column eluant was collected, 50 μL of PBS was added to the column, and the column was centrifuged again as described above, with the eluant being collected into a new 13 x 100 mm tube. This was repeated until a total of 36 fractions had been collected. Each fraction was assayed for lipid and DNA after the addition of 250 μL of distilled water. Lipid was assayed by scintillation counting or phosphate analysis (Fiske and Subbarow, 1925) of a 50 μL aliquot from each sample and the remainder (approximately 250 μl) was assayed for DNA by a Bligh-Dyer extraction protocol. The results are shown in Figure 1.

1.4 Extraction Protocol

DNA in 250 μL H_2O was separated from the lipid by the addition of 750 μL of chloroform:methanol (1:2.1) to form a single phase consisting of chloroform:methanol:H_2O (1:2.1:1). Additional volumes of H_2O (250 μL) and chloroform (250 μL) were added to the sample, resulting in a two phase system. The sample was then centrifuged at 3000 rpm for 10 min to facilitate rapid separation of the organic and aqueous layers. The upper, aqueous, phase was collected and 400 μL was assayed for DNA by absorbance at 260 nm.

The lower, chloroform, phase was washed three times with 300 μL of methanol:H_2O (1:1) and dried under N_2. The lipid film was hydrated in 1 mL of H_2O and an aliquot was taken for phosphate assay.

1.5 Stability of the Encapsulated Liposomal Antisense

Since the liposomal antisense preparation was not always used immediately it was necessary to analyze the stability of the preparation over several days. To this end, retention of antisense in liposomes was measured over a 5 day period, at 4°C and at room temperature. Leakage of entrapped antisense would appear as a peak in the included volume of Biogel A15m spin columns. At various times, 50 μL aliquots of the sample were applied to a 1 mL Biogel A15m column and eluted as described above. Lipid and oligonucleotide were determined as described above. A second peak in the oligonucleotide elution profile was taken to indicate leakage of entrapped oligonucleotide.

No leakage was indicated, either at 4°C or room temperature, even after 5 days (see Figures 3 and 4).

EXAMPLE 2

This example illustrates the complement activation by a liposomal antisense formulation.

To monitor the effects of various liposome and/or DNA formulations we have established an *in vitro* complement hemolytic assay using human serum for screening the relative complement activating properties of these formulations. The assay is a two-step procedure. The first step involves consumption of complement by liposomes and/or DNA, while the second step involves the lysis of antibody-sensitized sheep red cells by any residual complement that may not have been activated in the first part of the assay.

Complement activation by liposomes and free antisense was investigated using EPC/CH liposome compositions of 100 ± 30 nm diameter.

2.1 Normal Human Serum Pool

The first component of the assay that was tested was the activity of the fresh serum pool. This should be tested each time a new serum pool is generated as there will be some differences in complement activity between serum pools which can affect the sensitivity of the assay. A series of serum dilutions was tested to determine what dilution would give both maximal red cell lysis and minimal interference in absorbance readings (the more concentrated the serum dilution the more background absorbance is observed). Anything less than a 100-fold serum dilution gave reasonable levels of red cell lysis. EDTA-GVB^{2-} was added at the end of the assay to inhibit complement activity. The volume of EDTA-GVB^{2-} can be modified to increase or decrease the absorbance range of the assay depending on the activity of the serum pool. For all subsequent assays a 50-fold serum dilution was used (25-fold dilution in step 1 and 2-fold dilution in step 2). Furthermore, the amount of EDTA-GVB^{2-} used to stop the assay was 1.0 mL, giving an absorbance range (A_{410}) of 0 - 0.8.

Blood from seven healthy males and six healthy females was gathered into chilled serum tubes and immediately placed in an ice/water bath. Thirty mL of blood was collected per individual. Tubes were centrifuged at 2500 rpm for 10 min at 4°C, every six tubes (to avoid clotting). Plasma was removed from all tubes and pooled into a 250 mL beaker, on ice. The pooled plasma was then incubated at 37°C for 30 min, in the presence of several cloning sticks (to help recess the clot). The clot was removed and recessed, generating approximately 100 mL of serum. The serum was aliquoted (1.0 mL) into 1.5 mL Eppendorf tubes and stored at -65°C until use.

2.2 Preparation of Sensitized Sheep Red Cells (EA cells)

An aliquot of whole sheep blood (a 50% solution in Alsever's; Cedarlane) was withdrawn from the stock solution and centrifuged for 10 min at 1500 rpm. The cells were then washed three times with 10 volumes of EDTA-GVB^{2-}. The washed cells were resuspended in a volume of EDTA-GVB^{2-} that is approximately 5-times that of the initial aliquot. An aliquot (100 μL) of the resuspended cells was mixed with 2.9 mL of distilled water in a cuvette and the absorbance at 541 nm was measured. The concentration of the cells was adjusted to 1 x 10^9 cells/mL with EDTA-GVB^{2-} according to the following information:

[RBC] (cells/mL)	O.D.	λ (nm)
1 x 10^9	0.385	541
5 x 10^8	0.192	541
2 x 10^8	0.654	414
1 x 10^8	0.327	414

The cell suspension was warmed to 37°C in a shaking bath and rabbit anti-sheep red blood cell antibody (hemolysin) was added to give a final antibody dilution of 1/500 (i.e. 20 μL of antibody into 10 mL of cells). This mixture was incubated for 30 min at 37°C. Following the incubation, the cells were centrifuged at 1500 rpm for 5 min at 4°C, the supernatant removed, and the cells washed with EDTA-GVB^{2-}. The cells were then washed 2 times with $DGVB^{2+}$ in order to further remove any free antibody and to introduce cations into the cell suspension. Finally, the cell concentration was adjusted to

2×10^8 cells/mL with $DGVB^{2+}$ using the information given above. Cells were maintained at 4°C at all times, after preparation, and were used on the same day.

2.3 Complement Hemolytic Assay

All samples were serially diluted over a concentration range covering several orders of magnitude. Diluted liposome or oligonucleotide samples (100 μL) were added to 100 μL of normal human serum (NHS) that had been diluted 5-times in $DGVB^{2+}$. Samples were incubated for 30 min at 37°C and subsequently placed on ice. Ice-cold $DGVB^{2+}$ (300 μL) was added to each tube to dilute any complement not already consumed.

Samples (100 μL) of the diluted incubation mixture were added to 100 μL of EA cells or 100 μL of $DGVB^{2+}$ (for color blanks - these are usually required at liposome concentrations > 1 mM). The mixture was incubated for 30 min at 37°C and subsequently placed on ice. EDTA-GVB^{2-} (1.0 mL) was added to the sample to inhibit complement activity and the mixture was centrifuged for 5 min at 4°C and 1500 rpm. Aliquots of the supernatant (250 μL) were transferred to a microtiter plate, in triplicate, with care not to disturb the pelleted red cells. The absorbance of the supernatant was measured at 410 nm on an electronic plate reader.

Figure 5 depicts the complement activating ability of the liposome composition. As can be seen, the neutral EPC:CH liposomes showed no observable complement activation over the concentration ranges studied.

EXAMPLE 3

This example illustrates the treatment of mice with liposomal antisense compositions to reduce inflammation due to delayed type hypersensitivity (DTH).

A murine model of contact sensitivity, a form of delayed type hypersensitivity (DTH), has been established. This model involves the sensitization of the abdominal region of mice with a strong contact sensitizing agent, dinitrofluorobenzene (DNFB). Inflammation can then be induced at a later time (5 days after initial sensitization) by challenging the ear with a dilute solution DNFB. This model has been characterized with respect to several common features of inflammation; specifically,

edema (ear thickness measurements), vascular leak (liposome accumulation in inflamed ears); and cell infiltration (myeloperoxidase assays for neutrophils/monocytes or by prelabeling bone marrow cells and circulating leukocytes with [^{3}H]-thymidine). Furthermore, both inbred (BALB/c) and outbred (ICR) mice have been tested in this model, with similar patterns of inflammation being observed for both strains of mice.

3.1 Mice

Female BALB/c and ICR mice were obtained from Harley and Sprague Davis. BALB/c mice were used at 6-9 weeks of age, while ICR mice were used at 8-10 weeks of age. Each experimental group consists of four mice and the experiments were repeated at least twice.

3.2 Sensitization and Elicitation of Contact Sensitivity

Mice were sensitized by applying 25 μL of 0.5% 2,4-dinitro-1-fluorobenzene (DNFB) in acetone:olive oil (4:1) to the shaved abdominal wall for two consecutive days. Four days after the second application, mice were challenged on the dorsal surface of the left ear with 10 μL of 0.2% DNFB in acetone:olive oil (4:1). Mice received no treatment on the contralateral (right) ear. In some cases, control mice received 10 μL of vehicle on the dorsal surface of the left ear.

3.3 Evaluation of Ear Swelling

Ear thickness was measured immediately prior to ear challenge, and at various time intervals after DNFB challenge, using an engineer's micrometer (Mitutoyo, Tokyo, Japan). Increases in ear thickness measurements were determined by subtracting the pre-challenge from post-challenge measurements.

The progression of ear inflammation over a 3 day period for ICR (outbred) and BALB/c (inbred) mice is indicated in Figures 6 and 7, respectively. Erythema was evident almost immediately after ear challenge and gradually declined in intensity over the remainder of the study. ICR mice exhibited peak ear thickness at 24 hours after the induction of ear inflammation. Maximal ear thickness measurements were found to be 170 x 10^{-4} inches, corresponding to a 70% increase in ear thickness. Although ear

swelling gradually declines at 48 and 72 hours after inflammation initiation, ear measurements still have not returned to baseline thickness levels (90-100 x 10^{-4} inches).

BALB/c mice demonstrate peak ear thickness measurements between 24-48 hours after ear challenge. The maximal ear thickness measurements exhibited by these mice were 130 x 10^{-4} inches, which corresponds to an increase of ~50% over baseline values (75-85 x 10^{-4} inches).

3.4 Evaluation of Liposome Accumulation

Large unilamellar vesicles composed of distearylphosphatidylcholine (DSPC) and cholesterol (CH), at a 55:45 molar ratio, were prepared in HEPES-buffered saline (20 mM HEPES, 145 mM NaCl, pH 7.4) by extrusion through 2 stacked 100 nm polycarbonate filters. Liposomes contained a non-exchangeable radioactive lipid marker, [^{3}H]cholesterylhexadecylether (CHE). LUVs were administered at a dose of 100 mg/kg (200 μL; ~2 μCi of CHE/mouse) via the dorsal tail vein at 0, 24, 48, 72 hr after initiation of ear inflammation. Liposomes were allowed to circulate for 24 hr after taking ear measurements. Mice were then terminated and the ears were collected for analysis of liposome accumulation and cell infiltration.

Analyses of liposome accumulation were performed so as to give a relative indication of the degeneration of the vasculature during various stages of inflammation. Consequently, liposome accumulation was measured over the following time intervals: 0-24 hr, 24-48 hr, 48-72 hr (and in some cases 72-96 hr). For both strains of mice maximal liposome accumulation occurred during the first 24 hr (Figures 8 and 9). Thus, the most prominent changes in the vasculature likely occurred during the 0-24 hr time period.

3.5 Evaluation of Cell Infiltration

Cellular infiltration in inflamed and non-inflamed ears was assessed by an enzymatic assay for neutrophil (and monocyte) myeloperoxidase (MPO) activity, or by pre-labeling bone marrow cells and circulating leukocytes with [^{3}H]-methyl thymidine.

In order to extract MPO from the azurophilic granules of the cells rather harsh conditions were employed. Ears were finely minced with scissors and added to 2.0 mL of phosphate buffer containing 0.5% hexadecyltrimethylammonium bromide (HTAB) (50 mM potassium phosphate, 0.5% HTAB, pH 6.0). The sample was then homogenized

using a polytron homogenizer for 1 min (high output), and sonicated for 30 sec (power output, 4; 40% pulse). The sample was then divided into two 1.0 mL portions. One portion was digested, using Solvable, and analyzed for CHE by standard liquid scintillation counting. The second portion was freeze-thawed five times in liquid nitrogen and again sonicated for 30 sec (power output, 4; 40% pulse). The sample was then centrifuged for 10 min at 18 000 x g to remove cellular debris. The supernatant was removed and assayed for MPO activity by incubating 0.1 mL aliquots with 2.9 mL of substrate buffer (50 mM potassium phosphate, pH 6.0 containing 0.167 mg/mL o-dianisidine dihydrochloride and 0.0005% hydrogen peroxide). Absorbance at 460 nm was monitored for several minutes and, in most cases, ΔA_{460} was taken from the absorbances at 30 and 90 seconds. All solutions were maintained on ice as much as possible through the procedure. One unit of MPO activity is defined as the amount of enzyme that degrades 1 μmol of peroxide per minute at 25°C.

For experiments in which blood cells were prelabeled with [^{3}H]-methyl thymidine, all mice received a 500 μL i.p. injection [^{3}H]-methyl thymidine in sterile saline (1 μCi/g of body weight) 24 hours prior to challenging the ear with DNFB. Ears were removed after taking ear thickness measurements, digested with Solvable, and analyzed for labeled cells by standard liquid scintillation analysis. A relative estimate of cell infiltration was made by expressing the ratio of the radioactivity observed in the inflamed versus the non-inflamed ear. Figure 10 shows a time course MPO accumulation in inflamed versus non-inflamed ears. Peak MPO activity occurs at approximately 48 hr and returns to baseline levels by 96 hr. Based on the accumulation of radiolabeled blood cells (predominantly neutrophils, monocytes, and T-lymphocytes), cell accumulation peaks by 24 hr and remains relatively high over 72 hours (see Figure 11). The major difference in these two procedures is that the MPO assay primarily measures neutrophil accumulation (neutrophils have three times more MPO than monocytes), whereas the [^{3}H]-methyl thymidine procedure measures the influx of all cells equally. Neutrophil accumulation at sites of inflammation has been demonstrated to rise rapidly over the first 24 hours and to decrease almost as rapidly. From 24-48 hours, increased levels of monocytes and T-cells are observed at the inflammation site.

3.6 A Typical Inflammation Experiment

Figure 12 details a typical inflammation experiment involving ICR mice. Ear swelling begins to show increases at approximately 12 hr after ear challenge. Lipid accumulation begins almost immediately and consistently increases over 24 hr (the 6 hr, 12 hr, and 24 hr lipid accumulation time points in this figure were injected at t=0 hr). Finally, a lag period can be observed before the onset of cell infiltration, which begins to increase consistently after 6 hr, peaks at 24 hr, and then slowly declines over 72 hr.

EXAMPLE 4

This example illustrates the passive targeting of large unilamellar vesicles to sites of inflammation using a murine ear inflammation model.

Liposome accumulation appeared to be maximal during the 0-24 and 24-48 hr time periods after the onset of inflammation, corresponding to peak inflammatory events. After this, liposome accumulation decreased dramatically, corresponding to remodeling and repair of the "leaky" vasculature.

Of primary importance to the development of inflammation are the morphological and functional alterations that occur in dermal microvascular cells. When activated by cytokines, endothelial cells vasodilate, resulting in increased vascular blood flow to the region of inflammation. In addition, the blood vessels are stimulated to structurally remodel, thus enabling immune cells to extravasate from the vasculature and access the inflammation site. After such alterations, the endothelium is optimized for the infiltration of leukocytes and macromolecules to the site of inflammation. Consequently, it would be expected that relatively small vesicles, such as liposomes (100 nm diameter in our studies), would avidly move through the "leaky" vasculature and passively accumulate at sites of inflammation.

4.1 Methods

The mice used, as well as the sensitization and elicitation of contact sensitivity were carried out as described above in Example 3. Liposome accumulation was monitored over the first 24 hours of inflammation. LUVs were administered at a dose of 100 mg/kg (200 μL; ~2 μCi of CHE/mouse) via the dorsal tail vein immediately

after ear challenge with DNFB. At 6, 12, and 24 hr after ear challenge, mice were terminated. Ears were collected and analyzed as described above.

4.2 Liposome Accumulation at a Site of Inflammation

Liposome accumulation was examined during various stages of murine ear inflammation so as to give a relative indication of the ability of these vesicles to extravasate through the "inflamed" vasculature. This is of interest for the passive targeting of liposomal drugs, such as anti-ICAM-1 oligonucleotides and corticosteroids, to sites of inflammation. Liposome accumulation was measured over the following time intervals: 0-24 hr, 24-48 hr, 48-72 hr. For both ICR and BALB/c mice, maximal liposome accumulation occurred during the first 24 hr of inflammation. Thus, it appears that the most prominent structural changes to the vasculature occurred during the 0-24 hr time period. This is consistent with previous reports detailing vascular leakage of relatively small molecules and proteins, such as BSA.

4.3 Liposome Accumulation over the first 24 hr of Inflammation

Figure 13 depicts the accumulation of DSPC:CH liposomes, administered immediately after ear challenge (t=0 hr), over the initial 24 hr of inflammation. As might be expected, lipid steadily accumulated in the inflamed ear during the first 24 hr, corresponding to the remodeling of the vascular endothelium in the inflamed region. No such increases in lipid accumulation were observed for control ears (non-inflamed), since no remodeling of the endothelium has occurred.

As Figure 13 illustrates, passive accumulation of LUVs occurs at sites of inflammation. Liposome accumulation appears to be maximal during the 0-24 hr time periods (after the onset of inflammation). After this, accumulation decreases dramatically, corresponding to remodeling and repair of the "leaky" vasculature.

EXAMPLE 5

This example illustrates the plasma clearance and biodistribution of free and encapsulated oligonucleotides.

Mice were sensitized and challenged as described in Example 3. Fifteen minutes after ear challenge, various antisense formulations were administered by the lateral tail (200 μL) at an oligonucleotide dose of 50 mg/kg. Control mice were injected with PBS or saline. At various timepoints blood was withdrawn from the mice by cardiac puncture and collected into plasma tubes containing EDTA. An aliquot of whole blood was removed for analysis. The blood was centrifuged at 3000 rpm for 10 min and an aliquot of the plasma was counted by standard liquid scintillation analysis. Organs were collected, homogenized, digested and analyzed for the presence of radiolabeled antisense and radiolabeled lipid by standard liquid scintillation counting techniques.

The relative rates of clearance for free and encapsulated oligonucleotide are shown in Figure 14. The circulation half-life for free oligonucleotide (ISIS 2302) was very short (about 2.5 minutes). However, the formulation with antisense encapsulated in EPC:CH (55:45) liposomes had a much slower rate of clearance from the circulation. Instead of a circulation half-life of minutes, the encapsulated oligonucleotide had a half-life of about 8 hours. Thus, encapsulation increases the circulation half-life approximately 100-fold.

Figure 15 shows the tissue distribution of [^{3}H]-antisense in the liver, spleen, lung, and kidney after intravenous injection of oligonucleotide (50 mg/kg dose). The antisense molecule was rapidly cleared from the plasma with the majority of the dose distributing primarily to the liver and kidney. Only minor accumulation was observed in the spleen and lung. On the basis of organ weight, however, the kidney was the most efficient organ for antisense removal. This result is consistent with reports that indicate that the major route of elimination of free antisense from the body is by urinary excretion. Figure 16 demonstrates the biodistribution profiles for both the lipid and antisense portions of the encapsulated formulation. The liver was found to be the primary organ of accumulation for both antisense and lipid. Some accumulation was noted in the spleen with trace amounts being detected in the lungs and kidneys. This pattern of biodistribution is very similar to standard liposome biodistribution profiles but is significantly different from the kidney and liver tissue distribution exhibited by free oligonucleotide. Thus, the biodistribution of the lipid component determines the distribution of the antisense molecule as would be expected if the antisense molecule does not leak out of the liposomes.

EXAMPLE 6

This example illustrates the efficacy of mouse anti-ICAM oligonucleotide.

The efficacy of antisense oligonucleotide against mouse ICAM-1 mRNA was tested using the ear inflammation model described above (Example 3). The test oligonucleotide was developed by Isis Pharmaceuticals and is referred to as Isis 3082. The antisense is a 20 base (20 mer) phosphorothioate against a sequence in the untranslated 5'-region of murine ICAM-1 mRNA (see, Bennett, *et al.*, *J. Immunol.* **152**:3530-3540 (1994); Bennett, *et al.*, *Adv. Pharmacol.* **28**:1-43 (1994); and Chiang, *et al.*, *J. Biol. Chem.* **266**:18162-18171 (1991)). This oligonucleotide has recently been shown to exhibit activity in reducing heart allograft rejection in mice (Stepkowski, *et al.*, *J. Immunol.* **153**:5336-5346 (1994) and Stepkowski, *et al.*, *Transplant. Proc.* **27**:113 (1995) but had not been found effective in the DTH model (personal communication, F. Bennett, Isis).

Ear thickness in the mice was measured immediately prior to ear challenge and 24 hr after DNFB challenge using an engineer's micrometer (Mitutoyo, Tokyo, Japan). Increases in ear thickness measurements were determined by subtracting the pre-challenge measurements from the post-challenge measurements.

Fifteen minutes after the initiation of murine ear inflammation, various antisense formulations were administered at an oligonucleotide dose of 50 mg/kg (in 200 μL) via the dorsal tail vein. Inflammation control mice were injected with PBS. Ear measurements were taken 24 hours later to assess edema formation (ear swelling).

The effect of free anti-ICAM-1 oligonucleotides on the development of murine ear inflammation is indicated in Figure 17. As can be seen, each of the sets of mice (treated with either free ISIS 2302 or 3082) exhibited increased ear thickness measurements equivalent to the inflammation control mice which had been injected with PBS. Thus, a single dose (50 mg/kg) of free oligonucleotide was insufficient for preventing the development of ear edema.

Encapsulated antisense oligonucleotides were injected as described above and the results are shown in Figure 18. Increases in ear thickness were observed in mice

that received an injection of PBS. Similarly, mice treated with a single dose of free ISIS 3082 antisense, empty EPC:CH liposomes, or an encapsulated human ICAM-1 specific oligonucleotide (ISIS 2302) showed significant increases (>100%) in ear thickness. However, a murine ICAM-1 specific antisense molecule (ISI 3082) encapsulated in EPC:CH liposomes was able to significantly reduce ear edema, whether injected 30 min prior to or immediately after initiating inflammation. This result is comparable to the control in which mice were treated topically with corticosteroid (HBP).

In a separate experiment, the ear weights of the DNFB-challenged and untreated ear were compared for mice treated with a single dose of free ISIS 3082, encapsulated ISIS 3082, and PBS (see Figure 19). An approximate 2-fold increase in the weight of the challenged ear was observed for mice treated with either the free antisense molecule or PBS. However, little difference was observed between left and right ear weights of mice treated with encapsulated ISIS 3082, indicating a lack of edema.

All publications, patents and patent applications mentioned in this specification are herein incorporated by reference into the specification to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated herein by reference.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be obvious that certain changes and modifications may be practiced within the scope of the appended claims.

SEQUENCE LISTING

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(ii) TITLE OF INVENTION: Enhanced Efficacy of Liposomal Antisense

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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
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(A) APPLICATION NUMBER: US 08/657,753
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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2490 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 117..1949
(D) OTHER INFORMATION: /product= "human endothelial leukocyte adhesion molecule 1 (ELAM-1)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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CCAAAACGGA AAGTATTTCA AGCCTAAACC TTTGGGTGAA AAGAACTCTT GAAGTC          116

ATG ATT GCT TCA CAG TTT CTC TCA GCT CTC ACT TTG GTG CTT CTC ATT         164
Met Ile Ala Ser Gln Phe Leu Ser Ala Leu Thr Leu Val Leu Leu Ile
 1               5                  10                  15

AAA GAG AGT GGA GCC TGG TCT TAC AAC ACC TCC ACG GAA GCT ATG ACT         212
Lys Glu Ser Gly Ala Trp Ser Tyr Asn Thr Ser Thr Glu Ala Met Thr
            20                  25                  30

TAT GAT GAG GCC AGT GCT TAT TGT CAG CAA AGG TAC ACA CAC CTG GTT         260
Tyr Asp Glu Ala Ser Ala Tyr Cys Gln Gln Arg Tyr Thr His Leu Val
        35                  40                  45

GCA ATT CAA AAC AAA GAA GAG ATT GAG TAC CTA AAC TCC ATA TTG AGC         308
Ala Ile Gln Asn Lys Glu Glu Ile Glu Tyr Leu Asn Ser Ile Leu Ser
    50                  55                  60

TAT TCA CCA AGT TAT TAC TGG ATT GGA ATC AGA AAA GTC AAC AAT GTG         356
Tyr Ser Pro Ser Tyr Tyr Trp Ile Gly Ile Arg Lys Val Asn Asn Val
65                  70                  75                  80

TGG GTC TGG GTA GGA ACC CAG AAA CCT CTG ACA GAA GAA GCC AAG AAC         404
Trp Val Trp Val Gly Thr Gln Lys Pro Leu Thr Glu Glu Ala Lys Asn
                85                  90                  95

TGG GCT CCA GGT GAA CCC AAC AAT AGG CAA AAA GAT GAG GAC TGC GTG         452
Trp Ala Pro Gly Glu Pro Asn Asn Arg Gln Lys Asp Glu Asp Cys Val
            100                 105                 110

GAG ATC TAC ATC AAG AGA GAA AAA GAT GTG GGC ATG TGG AAT GAT GAG         500
Glu Ile Tyr Ile Lys Arg Glu Lys Asp Val Gly Met Trp Asn Asp Glu
        115                 120                 125

AGG TGC AGC AAG AAG AAG CTT GCC CTA TGC TAC ACA GCT GCC TGT ACC         548
Arg Cys Ser Lys Lys Lys Leu Ala Leu Cys Tyr Thr Ala Ala Cys Thr
    130                 135                 140

AAT ACA TCC TGC AGT GGC CAC GGT GAA TGT GTA GAG ACC ATC AAT AAT         596
Asn Thr Ser Cys Ser Gly His Gly Glu Cys Val Glu Thr Ile Asn Asn
145                 150                 155                 160

TAC ACT TGC AAG TGT GAC CCT GGC TTC AGT GGA CTC AAG TGT GAG CAA         644
Tyr Thr Cys Lys Cys Asp Pro Gly Phe Ser Gly Leu Lys Cys Glu Gln
                165                 170                 175

ATT GTG AAC TGT ACA GCC CTG GAA TCC CCT GAG CAT GGA AGC CTG GTT         692
Ile Val Asn Cys Thr Ala Leu Glu Ser Pro Glu His Gly Ser Leu Val
            180                 185                 190

TGC AGT CAC CCA CTG GGA AAC TTC AGC TAC AAT TCT TCC TGC TCT ATC         740
Cys Ser His Pro Leu Gly Asn Phe Ser Tyr Asn Ser Ser Cys Ser Ile
        195                 200                 205

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Ser Cys Asp Arg Gly Tyr Leu Pro Ser Ser Met Glu Thr Met Gln Cys
    210                 215                 220
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Met Ser Ser Gly Glu Trp Ser Ala Pro Ile Pro Ala Cys Asn Val Val
225                 230                 235                 240

GAG TGT GAT GCT GTG ACA AAT CCA GCC AAT GGG TTC GTG GAA TGT TTC        884
Glu Cys Asp Ala Val Thr Asn Pro Ala Asn Gly Phe Val Glu Cys Phe
                245                 250                 255

CAA AAC CCT GGA AGC TTC CCA TGG AAC ACA ACC TGT ACA TTT GAC TGT        932
Gln Asn Pro Gly Ser Phe Pro Trp Asn Thr Thr Cys Thr Phe Asp Cys
            260                 265                 270

GAA GAA GGA TTT GAA CTA ATG GGA GCC CAG AGC CTT CAG TGT ACC TCA        980
Glu Glu Gly Phe Glu Leu Met Gly Ala Gln Ser Leu Gln Cys Thr Ser
        275                 280                 285

TCT GGG AAT TGG GAC AAC GAG AAG CCA ACG TGT AAA GCT GTG ACA TGC       1028
Ser Gly Asn Trp Asp Asn Glu Lys Pro Thr Cys Lys Ala Val Thr Cys
    290                 295                 300

AGG GCC GTC CGC CAG CCT CAG AAT GGC TCT GTG AGG TGC AGC CAT TCC       1076
Arg Ala Val Arg Gln Pro Gln Asn Gly Ser Val Arg Cys Ser His Ser
305                 310                 315                 320

CCT GCT GGA GAG TTC ACC TTC AAA TCA TCC TGC AAC TTC ACC TGT GAG       1124
Pro Ala Gly Glu Phe Thr Phe Lys Ser Ser Cys Asn Phe Thr Cys Glu
                325                 330                 335

GAA GGC TTC ATG TTG CAG GGA CCA GCC CAG GTT GAA TGC ACC ACT CAA       1172
Glu Gly Phe Met Leu Gln Gly Pro Ala Gln Val Glu Cys Thr Thr Gln
            340                 345                 350

GGG CAG TGG ACA CAG CAA ATC CCA GTT TGT GAA GCT TTC CAG TGC ACA       1220
Gly Gln Trp Thr Gln Gln Ile Pro Val Cys Glu Ala Phe Gln Cys Thr
        355                 360                 365

GCC TTG TCC AAC CCC GAG CGA GGC TAC ATG AAT TGT CTT CCT AGT GCT       1268
Ala Leu Ser Asn Pro Glu Arg Gly Tyr Met Asn Cys Leu Pro Ser Ala
    370                 375                 380

TCT GGC AGT TTC CGT TAT GGG TCC AGC TGT GAG TTC TCC TGT GAG CAG       1316
Ser Gly Ser Phe Arg Tyr Gly Ser Ser Cys Glu Phe Ser Cys Glu Gln
385                 390                 395                 400

GGT TTT GTG TTG AAG GGA TCC AAA AGG CTC CAA TGT GGC CCC ACA GGG       1364
Gly Phe Val Leu Lys Gly Ser Lys Arg Leu Gln Cys Gly Pro Thr Gly
                405                 410                 415

GAG TGG GAC AAC GAG AAG CCC ACA TGT GAA GCT GTG AGA TGC GAT GCT       1412
Glu Trp Asp Asn Glu Lys Pro Thr Cys Glu Ala Val Arg Cys Asp Ala
            420                 425                 430

GTC CAC CAG CCC CCG AAG GGT TTG GTG AGG TGT GCT CAT TCC CCT ATT       1460
Val His Gln Pro Pro Lys Gly Leu Val Arg Cys Ala His Ser Pro Ile
        435                 440                 445

GGA GAA TTC ACC TAC AAG TCC TCT TGT GCC TTC AGC TGT GAG GAG GGA       1508
Gly Glu Phe Thr Tyr Lys Ser Ser Cys Ala Phe Ser Cys Glu Glu Gly
    450                 455                 460

TTT GAA TTA TAT GGA TCA ACT CAA CTT GAG TGC ACA TCT CAG GGA CAA       1556
Phe Glu Leu Tyr Gly Ser Thr Gln Leu Glu Cys Thr Ser Gln Gly Gln
465                 470                 475                 480

TGG ACA GAA GAG GTT CCT TCC TGC CAA GTG GTA AAA TGT TCA AGC CTG       1604
Trp Thr Glu Glu Val Pro Ser Cys Gln Val Val Lys Cys Ser Ser Leu
                485                 490                 495
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GCA GTT CCG GGA AAG ATC AAC ATG AGC TGC AGT GGG GAG CCC GTG TTT      1652
Ala Val Pro Gly Lys Ile Asn Met Ser Cys Ser Gly Glu Pro Val Phe
            500                 505                 510

GGC ACT GTG TGC AAG TTC GCC TGT CCT GAA GGA TGG ACG CTC AAT GGC      1700
Gly Thr Val Cys Lys Phe Ala Cys Pro Glu Gly Trp Thr Leu Asn Gly
        515                 520                 525

TCT GCA GCT CGG ACA TGT GGA GCC ACA GGA CAC TGG TCT GGC CTG CTA      1748
Ser Ala Ala Arg Thr Cys Gly Ala Thr Gly His Trp Ser Gly Leu Leu
    530                 535                 540

CCT ACC TGT GAA GCT CCC ACT GAG TCC AAC ATT CCC TTG GTA GCT GGA      1796
Pro Thr Cys Glu Ala Pro Thr Glu Ser Asn Ile Pro Leu Val Ala Gly
545                 550                 555                 560

CTT TCT GCT GCT GGA CTC TCC CTC CTG ACA TTA GCA CCA TTT CTC CTC      1844
Leu Ser Ala Ala Gly Leu Ser Leu Leu Thr Leu Ala Pro Phe Leu Leu
                565                 570                 575

TGG CTT CGG AAA TGC TTA CGG AAA GCA AAG AAA TTT GTT CCT GCC AGC      1892
Trp Leu Arg Lys Cys Leu Arg Lys Ala Lys Lys Phe Val Pro Ala Ser
            580                 585                 590

AGC TGC CAA AGC CTT GAA TCA GAC GGA AGC TAC CAA AAG CCT TCT TAC      1940
Ser Cys Gln Ser Leu Glu Ser Asp Gly Ser Tyr Gln Lys Pro Ser Tyr
        595                 600                 605

ATC CTT TAAGTTCAAA AGAATCAGAA ACAGGTGCAT CTGGGGAACT AGAGGGATAC      1996
Ile Leu
    610

ACTGAAGTTA ACAGAGACAG ATAACTCTCC TCGGGTCTCT GGCCCTTCTT GCCTACTATG   2056

CCAGATGCCT TTATGGCTGA AACCGCAACA CCCATCACCA CTTCAATAGA TCAAAGTCCA   2116

GCAGGCAAGG ACGGCCTTCA ACTGAAAAGA CTCAGTGTTC CCTTTCCTAC TCTCAGGATC   2176

AAGAAAGTGT TGGCTAATGA AGGGAAAGGA TATTTTCTTC CAAGCAAAGG TGAAGAGACC   2236

AAGACTCTGA AATCTCAGAA TTCCTTTTCT AACTCTCCCT TGCTCGCTGT AAAATCTTGG   2296

CACAGAAACA CAATATTTTG TGGCTTTCTT TCTTTTGCCC TTCACAGTGT TTCGACAGCT   2356

GATTACACAG TTGCTGTCAT AAGAATGAAT AATAATTATC CAGAGTTTAG AGGAAAAAAA   2416

TGACTAAAAA TATTATAACT TAAAAAAATG ACAGATGTTG AATGCCCACA GGCAAATGCA   2476

TGGAGGGTTG TTAA                                                     2490
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 610 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Met Ile Ala Ser Gln Phe Leu Ser Ala Leu Thr Leu Val Leu Leu Ile
  1               5                  10                  15

Lys Glu Ser Gly Ala Trp Ser Tyr Asn Thr Ser Thr Glu Ala Met Thr
            20                  25                  30
```

```
Tyr Asp Glu Ala Ser Ala Tyr Cys Gln Gln Arg Tyr Thr His Leu Val
        35                  40                  45

Ala Ile Gln Asn Lys Glu Glu Ile Glu Tyr Leu Asn Ser Ile Leu Ser
    50                  55                  60

Tyr Ser Pro Ser Tyr Tyr Trp Ile Gly Ile Arg Lys Val Asn Asn Val
65                  70                  75                  80

Trp Val Trp Val Gly Thr Gln Lys Pro Leu Thr Glu Glu Ala Lys Asn
                85                  90                  95

Trp Ala Pro Gly Glu Pro Asn Asn Arg Gln Lys Asp Glu Asp Cys Val
            100                 105                 110

Glu Ile Tyr Ile Lys Arg Glu Lys Asp Val Gly Met Trp Asn Asp Glu
        115                 120                 125

Arg Cys Ser Lys Lys Lys Leu Ala Leu Cys Tyr Thr Ala Ala Cys Thr
    130                 135                 140

Asn Thr Ser Cys Ser Gly His Gly Glu Cys Val Glu Thr Ile Asn Asn
145                 150                 155                 160

Tyr Thr Cys Lys Cys Asp Pro Gly Phe Ser Gly Leu Lys Cys Glu Gln
                165                 170                 175

Ile Val Asn Cys Thr Ala Leu Glu Ser Pro Glu His Gly Ser Leu Val
            180                 185                 190

Cys Ser His Pro Leu Gly Asn Phe Ser Tyr Asn Ser Ser Cys Ser Ile
        195                 200                 205

Ser Cys Asp Arg Gly Tyr Leu Pro Ser Ser Met Glu Thr Met Gln Cys
    210                 215                 220

Met Ser Ser Gly Glu Trp Ser Ala Pro Ile Pro Ala Cys Asn Val Val
225                 230                 235                 240

Glu Cys Asp Ala Val Thr Asn Pro Ala Asn Gly Phe Val Glu Cys Phe
                245                 250                 255

Gln Asn Pro Gly Ser Phe Pro Trp Asn Thr Thr Cys Thr Phe Asp Cys
            260                 265                 270

Glu Glu Gly Phe Glu Leu Met Gly Ala Gln Ser Leu Gln Cys Thr Ser
        275                 280                 285

Ser Gly Asn Trp Asp Asn Glu Lys Pro Thr Cys Lys Ala Val Thr Cys
    290                 295                 300

Arg Ala Val Arg Gln Pro Gln Asn Gly Ser Val Arg Cys Ser His Ser
305                 310                 315                 320

Pro Ala Gly Glu Phe Thr Phe Lys Ser Ser Cys Asn Phe Thr Cys Glu
                325                 330                 335

Glu Gly Phe Met Leu Gln Gly Pro Ala Gln Val Glu Cys Thr Thr Gln
            340                 345                 350

Gly Gln Trp Thr Gln Gln Ile Pro Val Cys Glu Ala Phe Gln Cys Thr
        355                 360                 365

Ala Leu Ser Asn Pro Glu Arg Gly Tyr Met Asn Cys Leu Pro Ser Ala
    370                 375                 380
```

```
Ser Gly Ser Phe Arg Tyr Gly Ser Ser Cys Glu Phe Ser Cys Glu Gln
385                 390                 395                 400

Gly Phe Val Leu Lys Gly Ser Lys Arg Leu Gln Cys Gly Pro Thr Gly
                405                 410                 415

Glu Trp Asp Asn Glu Lys Pro Thr Cys Glu Ala Val Arg Cys Asp Ala
            420                 425                 430

Val His Gln Pro Pro Lys Gly Leu Val Arg Cys Ala His Ser Pro Ile
        435                 440                 445

Gly Glu Phe Thr Tyr Lys Ser Ser Cys Ala Phe Ser Cys Glu Glu Gly
    450                 455                 460

Phe Glu Leu Tyr Gly Ser Thr Gln Leu Glu Cys Thr Ser Gln Gly Gln
465                 470                 475                 480

Trp Thr Glu Glu Val Pro Ser Cys Gln Val Val Lys Cys Ser Ser Leu
                485                 490                 495

Ala Val Pro Gly Lys Ile Asn Met Ser Cys Ser Gly Glu Pro Val Phe
            500                 505                 510

Gly Thr Val Cys Lys Phe Ala Cys Pro Glu Gly Trp Thr Leu Asn Gly
        515                 520                 525

Ser Ala Ala Arg Thr Cys Gly Ala Thr Gly His Trp Ser Gly Leu Leu
    530                 535                 540

Pro Thr Cys Glu Ala Pro Thr Glu Ser Asn Ile Pro Leu Val Ala Gly
545                 550                 555                 560

Leu Ser Ala Ala Gly Leu Ser Leu Leu Thr Leu Ala Pro Phe Leu Leu
                565                 570                 575

Trp Leu Arg Lys Cys Leu Arg Lys Ala Lys Lys Phe Val Pro Ala Ser
            580                 585                 590

Ser Cys Gln Ser Leu Glu Ser Asp Gly Ser Tyr Gln Lys Pro Ser Tyr
        595                 600                 605

Ile Leu
    610
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2220 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..2220
(D) OTHER INFORMATION: /product= "human vascular cell adhesion molecule 1 (VCAM-1)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
ATG CCT GGG AAG ATG GTC GTG ATC CTT GGA GCC TCA AAT ATA CTT TGG      48
Met Pro Gly Lys Met Val Val Ile Leu Gly Ala Ser Asn Ile Leu Trp
  1               5                  10                  15

ATA ATG TTT GCA GCT TCT CAA GCT TTT AAA ATC GAG ACC ACC CCA GAA      96
Ile Met Phe Ala Ala Ser Gln Ala Phe Lys Ile Glu Thr Thr Pro Glu
            20                  25                  30

TCT AGA TAT CTT GCT CAG ATT GGT GAC TCC GTC TCA TTG ACT TGC AGC     144
Ser Arg Tyr Leu Ala Gln Ile Gly Asp Ser Val Ser Leu Thr Cys Ser
        35                  40                  45

ACC ACA GGC TGT GAG TCC CCA TTT TTC TCT TGG AGA ACC CAG ATA GAT     192
Thr Thr Gly Cys Glu Ser Pro Phe Phe Ser Trp Arg Thr Gln Ile Asp
    50                  55                  60

AGT CCA CTG AAT GGG AAG GTG ACG AAT GAG GGG ACC ACA TCT ACG CTG     240
Ser Pro Leu Asn Gly Lys Val Thr Asn Glu Gly Thr Thr Ser Thr Leu
65                  70                  75                  80

ACA ATG AAT CCT GTT AGT TTT GGG AAC GAA CAC TCT TAC CTG TGC ACA     288
Thr Met Asn Pro Val Ser Phe Gly Asn Glu His Ser Tyr Leu Cys Thr
                85                  90                  95

GCA ACT TGT GAA TCT AGG AAA TTG GAA AAA GGA ATC CAG GTG GAG ATC     336
Ala Thr Cys Glu Ser Arg Lys Leu Glu Lys Gly Ile Gln Val Glu Ile
            100                 105                 110

TAC TCT TTT CCT AAG GAT CCA GAG ATT CAT TTG AGT GGC CCT CTG GAG     384
Tyr Ser Phe Pro Lys Asp Pro Glu Ile His Leu Ser Gly Pro Leu Glu
        115                 120                 125

GCT GGG AAG CCG ATC ACA GTC AAG TGT TCA GTT GCT GAT GTA TAC CCA     432
Ala Gly Lys Pro Ile Thr Val Lys Cys Ser Val Ala Asp Val Tyr Pro
    130                 135                 140

TTT GAC AGG CTG GAG ATA GAC TTA CTG AAA GGA GAT CAT CTC ATG AAG     480
Phe Asp Arg Leu Glu Ile Asp Leu Leu Lys Gly Asp His Leu Met Lys
145                 150                 155                 160

AGT CAG GAA TTT CTG GAG GAT GCA GAC AGG AAG TCC CTG GAA ACC AAG     528
Ser Gln Glu Phe Leu Glu Asp Ala Asp Arg Lys Ser Leu Glu Thr Lys
                165                 170                 175

AGT TTG GAA GTA ACC TTT ACT CCT GTC ATT GAG GAT ATT GGA AAA GTT     576
Ser Leu Glu Val Thr Phe Thr Pro Val Ile Glu Asp Ile Gly Lys Val
            180                 185                 190

CTT GTT TGC CGA GCT AAA TTA CAC ATT GAT GAA ATG GAT TCT GTG CCC     624
Leu Val Cys Arg Ala Lys Leu His Ile Asp Glu Met Asp Ser Val Pro
        195                 200                 205

ACA GTA AGG CAG GCT GTA AAA GAA TTG CAA GTC TAC ATA TCA CCC AAG     672
Thr Val Arg Gln Ala Val Lys Glu Leu Gln Val Tyr Ile Ser Pro Lys
    210                 215                 220

AAT ACA GTT ATT TCT GTG AAT CCA TCC ACA AAG CTG CAA GAA GGT GGC     720
Asn Thr Val Ile Ser Val Asn Pro Ser Thr Lys Leu Gln Glu Gly Gly
225                 230                 235                 240

TCT GTG ACC ATG ACC TGT TCC AGC GAG GGT CTA CCA GCT CCA GAG ATT     768
Ser Val Thr Met Thr Cys Ser Ser Glu Gly Leu Pro Ala Pro Glu Ile
                245                 250                 255
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TTC TGG AGT AAG AAA TTA GAT AAT GGG AAT CTA CAG CAC CTT TCT GGA          816
Phe Trp Ser Lys Lys Leu Asp Asn Gly Asn Leu Gln His Leu Ser Gly
            260                 265                 270

AAT GCA ACT CTC ACC TTA ATT GCT ATG AGG ATG GAA GAT TCT GGA ATT          864
Asn Ala Thr Leu Thr Leu Ile Ala Met Arg Met Glu Asp Ser Gly Ile
        275                 280                 285

TAT GTG TGT GAA GGA GTT AAT TTG ATT GGG AAA AAC AGA AAA GAG GTG          912
Tyr Val Cys Glu Gly Val Asn Leu Ile Gly Lys Asn Arg Lys Glu Val
    290                 295                 300

GAA TTA ATT GTT CAA GAG AAA CCA TTT ACT GTT GAG ATC TCC CCT GGA          960
Glu Leu Ile Val Gln Glu Lys Pro Phe Thr Val Glu Ile Ser Pro Gly
305                 310                 315                 320

CCC CGG ATT GCT GCT CAG ATT GGA GAC TCA GTC ATG TTG ACA TGT AGT         1008
Pro Arg Ile Ala Ala Gln Ile Gly Asp Ser Val Met Leu Thr Cys Ser
                325                 330                 335

GTC ATG GGC TGT GAA TCC CCA TCT TTC TCC TGG AGA ACC CAG ATA GAC         1056
Val Met Gly Cys Glu Ser Pro Ser Phe Ser Trp Arg Thr Gln Ile Asp
            340                 345                 350

AGC CCT CTG AGC GGG AAG GTG AGG AGT GAG GGG ACC AAT TCC ACG CTG         1104
Ser Pro Leu Ser Gly Lys Val Arg Ser Glu Gly Thr Asn Ser Thr Leu
        355                 360                 365

ACC CTG AGC CCT GTG AGT TTT GAG AAC GAA CAC TCT TAT CTG TGC ACA         1152
Thr Leu Ser Pro Val Ser Phe Glu Asn Glu His Ser Tyr Leu Cys Thr
    370                 375                 380

GTG ACT TGT GGA CAT AAG AAA CTG GAA AAG GGA ATC CAG GTG GAG CTC         1200
Val Thr Cys Gly His Lys Lys Leu Glu Lys Gly Ile Gln Val Glu Leu
385                 390                 395                 400

TAC TCA TTC CCT AGA GAT CCA GAA ATC GAG ATG AGT GGT GGC CTC GTG         1248
Tyr Ser Phe Pro Arg Asp Pro Glu Ile Glu Met Ser Gly Gly Leu Val
                405                 410                 415

AAT GGG AGC TCT GTC ACT GTA AGC TGC AAG GTT CCT AGC GTG TAC CCC         1296
Asn Gly Ser Ser Val Thr Val Ser Cys Lys Val Pro Ser Val Tyr Pro
            420                 425                 430

CTT GAC CGG CTG GAG ATT GAA TTA CTT AAG GGG GAG ACT ATT CTG GAG         1344
Leu Asp Arg Leu Glu Ile Glu Leu Leu Lys Gly Glu Thr Ile Leu Glu
        435                 440                 445

AAT ATA GAG TTT TTG GAG GAT ACG GAT ATG AAA TCT CTA GAG AAC AAA         1392
Asn Ile Glu Phe Leu Glu Asp Thr Asp Met Lys Ser Leu Glu Asn Lys
    450                 455                 460

AGT TTG GAA ATG ACC TTC ATC CCT ACC ATT GAA GAT ACT GGA AAA GCT         1440
Ser Leu Glu Met Thr Phe Ile Pro Thr Ile Glu Asp Thr Gly Lys Ala
465                 470                 475                 480

CTT GTT TGT CAG GCT AAG TTA CAT ATT GAT GAC ATG GAA TTC GAA CCC         1488
Leu Val Cys Gln Ala Lys Leu His Ile Asp Asp Met Glu Phe Glu Pro
                485                 490                 495

AAA CAA AGG CAG AGT ACG CAA ACA CTT TAT GTC AAT GTT GCC CCC AGA         1536
Lys Gln Arg Gln Ser Thr Gln Thr Leu Tyr Val Asn Val Ala Pro Arg
            500                 505                 510

GAT ACA ACC GTC TTG GTC AGC CCT TCC TCC ATC CTG GAG GAA GGC AGT         1584
Asp Thr Thr Val Leu Val Ser Pro Ser Ser Ile Leu Glu Glu Gly Ser
        515                 520                 525
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TCT GTG AAT ATG ACA TGC TTG AGC CAG GGC TTT CCT GCT CCG AAA ATC      1632
Ser Val Asn Met Thr Cys Leu Ser Gln Gly Phe Pro Ala Pro Lys Ile
    530                 535                 540

CTG TGG AGC AGG CAG CTC CCT AAC GGG GAG CTA CAG CCT CTT TCT GAG      1680
Leu Trp Ser Arg Gln Leu Pro Asn Gly Glu Leu Gln Pro Leu Ser Glu
545                 550                 555                 560

AAT GCA ACT CTC ACC TTA ATT TCT ACA AAA ATG GAA GAT TCT GGG GTT      1728
Asn Ala Thr Leu Thr Leu Ile Ser Thr Lys Met Glu Asp Ser Gly Val
                565                 570                 575

TAT TTA TGT GAA GGA ATT AAC CAG GCT GGA AGA AGC AGA AAG GAA GTG      1776
Tyr Leu Cys Glu Gly Ile Asn Gln Ala Gly Arg Ser Arg Lys Glu Val
            580                 585                 590

GAA TTA ATT ATC CAA GTT ACT CCA AAA GAC ATA AAA CTT ACA GCT TTT      1824
Glu Leu Ile Ile Gln Val Thr Pro Lys Asp Ile Lys Leu Thr Ala Phe
        595                 600                 605

CCT TCT GAG AGT GTC AAA GAA GGA GAC ACT GTC ATC ATC TCT TGT ACA      1872
Pro Ser Glu Ser Val Lys Glu Gly Asp Thr Val Ile Ile Ser Cys Thr
    610                 615                 620

TGT GGA AAT GTT CCA GAA ACA TGG ATA ATC CTG AAG AAA AAA GCG GAG      1920
Cys Gly Asn Val Pro Glu Thr Trp Ile Ile Leu Lys Lys Lys Ala Glu
625                 630                 635                 640

ACA GGA GAC ACA GTA CTA AAA TCT ATA GAT GGC GCC TAT ACC ATC CGA      1968
Thr Gly Asp Thr Val Leu Lys Ser Ile Asp Gly Ala Tyr Thr Ile Arg
                645                 650                 655

AAG GCC CAG TTG AAG GAT GCG GGA GTA TAT GAA TGT GAA TCT AAA AAC      2016
Lys Ala Gln Leu Lys Asp Ala Gly Val Tyr Glu Cys Glu Ser Lys Asn
            660                 665                 670

AAA GTT GGC TCA CAA TTA AGA AGT TTA ACA CTT GAT GTT CAA GGA AGA      2064
Lys Val Gly Ser Gln Leu Arg Ser Leu Thr Leu Asp Val Gln Gly Arg
        675                 680                 685

GAA AAC AAC AAA GAC TAT TTT TCT CCT GAG CTT CTC GTG CTC TAT TTT      2112
Glu Asn Asn Lys Asp Tyr Phe Ser Pro Glu Leu Leu Val Leu Tyr Phe
    690                 695                 700

GCA TCC TCC TTA ATA ATA CCT GCC ATT GGA ATG ATA ATT TAC TTT GCA      2160
Ala Ser Ser Leu Ile Ile Pro Ala Ile Gly Met Ile Ile Tyr Phe Ala
705                 710                 715                 720

AGA AAA GCC AAC ATG AAG GGG TCA TAT AGT CTT GTA GAA GCA CAG AAA      2208
Arg Lys Ala Asn Met Lys Gly Ser Tyr Ser Leu Val Glu Ala Gln Lys
                725                 730                 735

TCA AAA GTG TAG                                                     2220
Ser Lys Val
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 739 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Pro Gly Lys Met Val Val Ile Leu Gly Ala Ser Asn Ile Leu Trp
1 5 10 15

Ile Met Phe Ala Ala Ser Gln Ala Phe Lys Ile Glu Thr Thr Pro Glu
20 25 30

Ser Arg Tyr Leu Ala Gln Ile Gly Asp Ser Val Ser Leu Thr Cys Ser
35 40 45

Thr Thr Gly Cys Glu Ser Pro Phe Phe Ser Trp Arg Thr Gln Ile Asp
50 55 60

Ser Pro Leu Asn Gly Lys Val Thr Asn Glu Gly Thr Thr Ser Thr Leu
65 70 75 80

Thr Met Asn Pro Val Ser Phe Gly Asn Glu His Ser Tyr Leu Cys Thr
85 90 95

Ala Thr Cys Glu Ser Arg Lys Leu Glu Lys Gly Ile Gln Val Glu Ile
100 105 110

Tyr Ser Phe Pro Lys Asp Pro Glu Ile His Leu Ser Gly Pro Leu Glu
115 120 125

Ala Gly Lys Pro Ile Thr Val Lys Cys Ser Val Ala Asp Val Tyr Pro
130 135 140

Phe Asp Arg Leu Glu Ile Asp Leu Leu Lys Gly Asp His Leu Met Lys
145 150 155 160

Ser Gln Glu Phe Leu Glu Asp Ala Asp Arg Lys Ser Leu Glu Thr Lys
165 170 175

Ser Leu Glu Val Thr Phe Thr Pro Val Ile Glu Asp Ile Gly Lys Val
180 185 190

Leu Val Cys Arg Ala Lys Leu His Ile Asp Glu Met Asp Ser Val Pro
195 200 205

Thr Val Arg Gln Ala Val Lys Glu Leu Gln Val Tyr Ile Ser Pro Lys
210 215 220

Asn Thr Val Ile Ser Val Asn Pro Ser Thr Lys Leu Gln Glu Gly Gly
225 230 235 240

Ser Val Thr Met Thr Cys Ser Ser Glu Gly Leu Pro Ala Pro Glu Ile
245 250 255

Phe Trp Ser Lys Lys Leu Asp Asn Gly Asn Leu Gln His Leu Ser Gly
260 265 270

Asn Ala Thr Leu Thr Leu Ile Ala Met Arg Met Glu Asp Ser Gly Ile
275 280 285

Tyr Val Cys Glu Gly Val Asn Leu Ile Gly Lys Asn Arg Lys Glu Val
290 295 300

Glu Leu Ile Val Gln Glu Lys Pro Phe Thr Val Glu Ile Ser Pro Gly
305 310 315 320

Pro Arg Ile Ala Ala Gln Ile Gly Asp Ser Val Met Leu Thr Cys Ser
325 330 335

Val Met Gly Cys Glu Ser Pro Ser Phe Ser Trp Arg Thr Gln Ile Asp
340 345 350

Ser Pro Leu Ser Gly Lys Val Arg Ser Glu Gly Thr Asn Ser Thr Leu
355 360 365

Thr Leu Ser Pro Val Ser Phe Glu Asn Glu His Ser Tyr Leu Cys Thr
370 375 380

Val Thr Cys Gly His Lys Lys Leu Glu Lys Gly Ile Gln Val Glu Leu
385 390 395 400

Tyr Ser Phe Pro Arg Asp Pro Glu Ile Glu Met Ser Gly Gly Leu Val
405 410 415

Asn Gly Ser Ser Val Thr Val Ser Cys Lys Val Pro Ser Val Tyr Pro
420 425 430

Leu Asp Arg Leu Glu Ile Glu Leu Leu Lys Gly Glu Thr Ile Leu Glu
435 440 445

Asn Ile Glu Phe Leu Glu Asp Thr Asp Met Lys Ser Leu Glu Asn Lys
450 455 460

Ser Leu Glu Met Thr Phe Ile Pro Thr Ile Glu Asp Thr Gly Lys Ala
465 470 475 480

Leu Val Cys Gln Ala Lys Leu His Ile Asp Asp Met Glu Phe Glu Pro
485 490 495

Lys Gln Arg Gln Ser Thr Gln Thr Leu Tyr Val Asn Val Ala Pro Arg
500 505 510

Asp Thr Thr Val Leu Val Ser Pro Ser Ser Ile Leu Glu Glu Gly Ser
515 520 525

Ser Val Asn Met Thr Cys Leu Ser Gln Gly Phe Pro Ala Pro Lys Ile
530 535 540

Leu Trp Ser Arg Gln Leu Pro Asn Gly Glu Leu Gln Pro Leu Ser Glu
545 550 555 560

Asn Ala Thr Leu Thr Leu Ile Ser Thr Lys Met Glu Asp Ser Gly Val
565 570 575

Tyr Leu Cys Glu Gly Ile Asn Gln Ala Gly Arg Ser Arg Lys Glu Val
580 585 590

Glu Leu Ile Ile Gln Val Thr Pro Lys Asp Ile Lys Leu Thr Ala Phe
595 600 605

Pro Ser Glu Ser Val Lys Glu Gly Asp Thr Val Ile Ile Ser Cys Thr
610 615 620

Cys Gly Asn Val Pro Glu Thr Trp Ile Ile Leu Lys Lys Lys Ala Glu
625 630 635 640

Thr Gly Asp Thr Val Leu Lys Ser Ile Asp Gly Ala Tyr Thr Ile Arg
645 650 655

Lys Ala Gln Leu Lys Asp Ala Gly Val Tyr Glu Cys Glu Ser Lys Asn
660 665 670

Lys Val Gly Ser Gln Leu Arg Ser Leu Thr Leu Asp Val Gln Gly Arg
675 680 685

Glu Asn Asn Lys Asp Tyr Phe Ser Pro Glu Leu Leu Val Leu Tyr Phe
690 695 700

```
Ala Ser Ser Leu Ile Ile Pro Ala Ile Gly Met Ile Ile Tyr Phe Ala
705                 710                 715                 720

Arg Lys Ala Asn Met Lys Gly Ser Tyr Ser Leu Val Glu Ala Gln Lys
                725                 730                 735

Ser Lys Val
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1846 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 13..1611
(D) OTHER INFORMATION: /product= "human intercellular adhesion molecule 1 (ICAM-1)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
CTCAGCCTCG CT ATG GCT CCC AGC AGC CCC CGG CCC GCG CTG CCC GCA          48
              Met Ala Pro Ser Ser Pro Arg Pro Ala Leu Pro Ala
                1               5                  10

CTC CTG GTC CTG CTC GGG GCT CTG TTC CCA GGA CCT GGC AAT GCC CAG        96
Leu Leu Val Leu Leu Gly Ala Leu Phe Pro Gly Pro Gly Asn Ala Gln
        15                  20                  25

ACA TCT GTG TCC CCC TCA AAA GTC ATC CTG CCC CGG GGA GGC TCC GTG       144
Thr Ser Val Ser Pro Ser Lys Val Ile Leu Pro Arg Gly Gly Ser Val
    30                  35                  40

CTG GTG ACA TGC AGC ACC TCC TGT GAC CAG CCC AAG TTG TTG GGC ATA       192
Leu Val Thr Cys Ser Thr Ser Cys Asp Gln Pro Lys Leu Leu Gly Ile
45                  50                  55                  60

GAG ACC CCG TTG CCT AAA AAG GAG TTG CTC CTG CCT GGG AAC AAC CGG       240
Glu Thr Pro Leu Pro Lys Lys Glu Leu Leu Leu Pro Gly Asn Asn Arg
                65                  70                  75

AAG GTG TAT GAA CTG AGC AAT GTG CAA GAA GAT AGC CAA CCA ATG TGC       288
Lys Val Tyr Glu Leu Ser Asn Val Gln Glu Asp Ser Gln Pro Met Cys
            80                  85                  90

TAT TCA AAC TGC CCT GAT GGG CAG TCA ACA GCT AAA ACC TTC CTC ACC       336
Tyr Ser Asn Cys Pro Asp Gly Gln Ser Thr Ala Lys Thr Phe Leu Thr
        95                  100                 105

GTG TAC TGG ACT CCA GAA CGG GTG GAA CTG GCA CCC CTC CCC TCT TGG       384
Val Tyr Trp Thr Pro Glu Arg Val Glu Leu Ala Pro Leu Pro Ser Trp
    110                 115                 120

CAG CCA GTG GGC AAG AAC CTT ACC CTA CGC TGC CAG GTG GAG GGT GGG       432
Gln Pro Val Gly Lys Asn Leu Thr Leu Arg Cys Gln Val Glu Gly Gly
125                 130                 135                 140
```

```
GCA CCC CGG GCC AAC CTC ACC GTG GTG CTG CTC CGT GGG GAG AAG GAG      480
Ala Pro Arg Ala Asn Leu Thr Val Val Leu Leu Arg Gly Glu Lys Glu
                145                 150                 155

CTG AAA CGG GAG CCA GCT GTG GGG GAG CCC GCT GAG GTC ACG ACC ACG      528
Leu Lys Arg Glu Pro Ala Val Gly Glu Pro Ala Glu Val Thr Thr Thr
            160                 165                 170

GTG CTG GTG AGG AGA GAT CAC CAT GGA GCC AAT TTC TCG TGC CGC ACT      576
Val Leu Val Arg Arg Asp His His Gly Ala Asn Phe Ser Cys Arg Thr
        175                 180                 185

GAA CTG GAC CTG CGG CCC CAA GGG CTG GAG CTG TTT GAG AAC ACC TCG      624
Glu Leu Asp Leu Arg Pro Gln Gly Leu Glu Leu Phe Glu Asn Thr Ser
    190                 195                 200

GCC CCC TAC CAG CTC CAG ACC TTT GTC CTG CCA GCG ACT CCC CCA CAA      672
Ala Pro Tyr Gln Leu Gln Thr Phe Val Leu Pro Ala Thr Pro Pro Gln
205                 210                 215                 220

CTT GTC AGC CCC CGG GTC CTA GAG GTG GAC ACG CAG GGG ACC GTG GTC      720
Leu Val Ser Pro Arg Val Leu Glu Val Asp Thr Gln Gly Thr Val Val
                225                 230                 235

TGT TCC CTG GAC GGG CTG TTC CCA GTC TCG GAG GCC CAG GTC CAC CTG      768
Cys Ser Leu Asp Gly Leu Phe Pro Val Ser Glu Ala Gln Val His Leu
            240                 245                 250

GCA CTG GGG GAC CAG AGG TTG AAC CCC ACA GTC ACC TAT GGC AAC GAC      816
Ala Leu Gly Asp Gln Arg Leu Asn Pro Thr Val Thr Tyr Gly Asn Asp
        255                 260                 265

TCC TTC TCG GCC AAG GCC TCA GTC AGT GTG ACC GCA GAG GAC GAG GGC      864
Ser Phe Ser Ala Lys Ala Ser Val Ser Val Thr Ala Glu Asp Glu Gly
    270                 275                 280

ACC CAG CGG CTG ACG TGT GCA GTA ATA CTG GGG AAC CAG AGC CAG GAG      912
Thr Gln Arg Leu Thr Cys Ala Val Ile Leu Gly Asn Gln Ser Gln Glu
285                 290                 295                 300

ACA CTG CAG ACA GTG ACC ATC TAC AGC TTT CCG GCG CCC AAC GTG ATT      960
Thr Leu Gln Thr Val Thr Ile Tyr Ser Phe Pro Ala Pro Asn Val Ile
                305                 310                 315

CTG ACG AAG CCA GAG GTC TCA GAA GGG ACC GAG GTG ACA GTG AAG TGT     1008
Leu Thr Lys Pro Glu Val Ser Glu Gly Thr Glu Val Thr Val Lys Cys
            320                 325                 330

GAG GCC CAC CCT AGA GCC AAG GTG ACG CTG AAT GGG GTT CCA GCC CAG     1056
Glu Ala His Pro Arg Ala Lys Val Thr Leu Asn Gly Val Pro Ala Gln
        335                 340                 345

CCA CTG GGC CCG AGG GCC CAG CTC CTG CTG AAG GCC ACC CCA GAG GAC     1104
Pro Leu Gly Pro Arg Ala Gln Leu Leu Leu Lys Ala Thr Pro Glu Asp
    350                 355                 360

AAC GGG CGC AGC TTC TCC TGC TCT GCA ACC CTG GAG GTG GCC GGC CAG     1152
Asn Gly Arg Ser Phe Ser Cys Ser Ala Thr Leu Glu Val Ala Gly Gln
365                 370                 375                 380

CTT ATA CAC AAG AAC CAG ACC CGG GAG CTT CGT GTC CTG TAT GGC CCC     1200
Leu Ile His Lys Asn Gln Thr Arg Glu Leu Arg Val Leu Tyr Gly Pro
                385                 390                 395

CGA CTG GAC GAG AGG GAT TGT CGG GGA AAC TGG ACG TGG CCA GAA AAT     1248
Arg Leu Asp Glu Arg Asp Cys Pro Gly Asn Trp Thr Trp Pro Glu Asn
            400                 405                 410
```

```
TCC CAG CAG ACT CCA ATG TGC CAG GCT TGG GGG AAC CCA TTG CCC GAG      1296
Ser Gln Gln Thr Pro Met Cys Gln Ala Trp Gly Asn Pro Leu Pro Glu
        415                 420                 425

CTC AAG TGT CTA AAG GAT GGC ACT TTC CCA CTG CCC ATC GGG GAA TCA      1344
Leu Lys Cys Leu Lys Asp Gly Thr Phe Pro Leu Pro Ile Gly Glu Ser
    430                 435                 440

GTG ACT GTC ACT CGA GAT CTT GAG GGC ACC TAC CTC TGT CGG GCC AGG      1392
Val Thr Val Thr Arg Asp Leu Glu Gly Thr Tyr Leu Cys Arg Ala Arg
445                 450                 455                 460

AGC ACT CAA GGG GAG GTC ACC CGC GAG GTG ACC GTG AAT GTG CTC TCC      1440
Ser Thr Gln Gly Glu Val Thr Arg Glu Val Thr Val Asn Val Leu Ser
                465                 470                 475

CCC CGG TAT GAG ATT GTC ATC ATC ACT GTG GTA GCA GCC GCA GTC ATA      1488
Pro Arg Tyr Glu Ile Val Ile Ile Thr Val Val Ala Ala Ala Val Ile
            480                 485                 490

ATG GGC ACT GCA GGC CTC AGC ACG TAC CTC TAT AAC CGC CAG CGG AAG      1536
Met Gly Thr Ala Gly Leu Ser Thr Tyr Leu Tyr Asn Arg Gln Arg Lys
        495                 500                 505

ATC AAG AAA TAC AGA CTA CAA CAG GCC CAA AAA GGG ACC CCC ATG AAA      1584
Ile Lys Lys Tyr Arg Leu Gln Gln Ala Gln Lys Gly Thr Pro Met Lys
    510                 515                 520

CCG AAC ACA CAA GCC ACG CCT CCC TGAACCTATC CCGGGACAGG GCCTCTTCCT     1638
Pro Asn Thr Gln Ala Thr Pro Pro
525                 530

CGGCCTTCCC ATATTGGTGG CAGTGGTGCC ACACTGAACA GAGTGGAAGA CATATGCCAT    1698

GCAGCTACAC CTACCGGCCC TGGGACGCCG GAGGACAGGG CATTGTCCTC AGTCAGATAC    1758

AACAGCATTT GGGGCCATGG TACCTGCACA CCTAAAACAC TAGGCCACGC ATCTGATCTG    1818

TAGTCACATG ACTAAGCCAA GAGGAAGG                                       1846
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 532 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
Met Ala Pro Ser Ser Pro Arg Pro Ala Leu Pro Ala Leu Leu Val Leu
  1               5                  10                  15

Leu Gly Ala Leu Phe Pro Gly Pro Gly Asn Ala Gln Thr Ser Val Ser
            20                  25                  30

Pro Ser Lys Val Ile Leu Pro Arg Gly Gly Ser Val Leu Val Thr Cys
        35                  40                  45

Ser Thr Ser Cys Asp Gln Pro Lys Leu Leu Gly Ile Glu Thr Pro Leu
    50                  55                  60

Pro Lys Lys Glu Leu Leu Leu Pro Gly Asn Asn Arg Lys Val Tyr Glu
65                  70                  75                  80
```

Leu Ser Asn Val Gln Glu Asp Ser Gln Pro Met Cys Tyr Ser Asn Cys
85 90 95

Pro Asp Gly Gln Ser Thr Ala Lys Thr Phe Leu Thr Val Tyr Trp Thr
100 105 110

Pro Glu Arg Val Glu Leu Ala Pro Leu Pro Ser Trp Gln Pro Val Gly
115 120 125

Lys Asn Leu Thr Leu Arg Cys Gln Val Glu Gly Gly Ala Pro Arg Ala
130 135 140

Asn Leu Thr Val Val Leu Leu Arg Gly Glu Lys Glu Leu Lys Arg Glu
145 150 155 160

Pro Ala Val Gly Glu Pro Ala Glu Val Thr Thr Thr Val Leu Val Arg
165 170 175

Arg Asp His His Gly Ala Asn Phe Ser Cys Arg Thr Glu Leu Asp Leu
180 185 190

Arg Pro Gln Gly Leu Glu Leu Phe Glu Asn Thr Ser Ala Pro Tyr Gln
195 200 205

Leu Gln Thr Phe Val Leu Pro Ala Thr Pro Pro Gln Leu Val Ser Pro
210 215 220

Arg Val Leu Glu Val Asp Thr Gln Gly Thr Val Val Cys Ser Leu Asp
225 230 235 240

Gly Leu Phe Pro Val Ser Glu Ala Gln Val His Leu Ala Leu Gly Asp
245 250 255

Gln Arg Leu Asn Pro Thr Val Thr Tyr Gly Asn Asp Ser Phe Ser Ala
260 265 270

Lys Ala Ser Val Ser Val Thr Ala Glu Asp Glu Gly Thr Gln Arg Leu
275 280 285

Thr Cys Ala Val Ile Leu Gly Asn Gln Ser Gln Glu Thr Leu Gln Thr
290 295 300

Val Thr Ile Tyr Ser Phe Pro Ala Pro Asn Val Ile Leu Thr Lys Pro
305 310 315 320

Glu Val Ser Glu Gly Thr Glu Val Thr Val Lys Cys Glu Ala His Pro
325 330 335

Arg Ala Lys Val Thr Leu Asn Gly Val Pro Ala Gln Pro Leu Gly Pro
340 345 350

Arg Ala Gln Leu Leu Leu Lys Ala Thr Pro Glu Asp Asn Gly Arg Ser
355 360 365

Phe Ser Cys Ser Ala Thr Leu Glu Val Ala Gly Gln Leu Ile His Lys
370 375 380

Asn Gln Thr Arg Glu Leu Arg Val Leu Tyr Gly Pro Arg Leu Asp Glu
385 390 395 400

Arg Asp Cys Pro Gly Asn Trp Thr Trp Pro Glu Asn Ser Gln Gln Thr
405 410 415

Pro Met Cys Gln Ala Trp Gly Asn Pro Leu Pro Glu Leu Lys Cys Leu
420 425 430

```
Lys Asp Gly Thr Phe Pro Leu Pro Ile Gly Glu Ser Val Thr Val Thr
        435                 440                 445

Arg Asp Leu Glu Gly Thr Tyr Leu Cys Arg Ala Arg Ser Thr Gln Gly
    450                 455                 460

Glu Val Thr Arg Glu Val Thr Val Asn Val Leu Ser Pro Arg Tyr Glu
465                 470                 475                 480

Ile Val Ile Ile Thr Val Val Ala Ala Ala Val Ile Met Gly Thr Ala
                485                 490                 495

Gly Leu Ser Thr Tyr Leu Tyr Asn Arg Gln Arg Lys Ile Lys Lys Tyr
            500                 505                 510

Arg Leu Gln Gln Ala Gln Lys Gly Thr Pro Met Lys Pro Asn Thr Gln
        515                 520                 525

Ala Thr Pro Pro
    530
```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2522 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 23..1636
(D) OTHER INFORMATION: /product= "mouse intercellular adhesion molecule 1 (ICAM-1)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
AGCACTCTGC CCTGGCCCTG CA ATG GCT TCA ACC CGT GCC AAG CCC ACG CTA        52
                         Met Ala Ser Thr Arg Ala Lys Pro Thr Leu
                           1               5                  10

CCT CTG CTC CTG GCC CTG GTC ACC GTT GTG ATC CCT GGG CCT GGT GAT        100
Pro Leu Leu Leu Ala Leu Val Thr Val Val Ile Pro Gly Pro Gly Asp
                  15                  20                  25

GCT CAG GTA TCC ATC CAT CCC AGA GAA GCC TTC CTG CCC CAG GGT GGG        148
Ala Gln Val Ser Ile His Pro Arg Glu Ala Phe Leu Pro Gln Gly Gly
              30                  35                  40

TCC GTG CAG GTG AAC TGT TCT TCC TCA TGC AAG GAG GAC CTC AGC CTG        196
Ser Val Gln Val Asn Cys Ser Ser Ser Cys Lys Glu Asp Leu Ser Leu
          45                  50                  55

GGC TTG GAG ACT CAG TGG CTG AAA GAT GAG CTC GAG AGT GGA CCC AAC        244
Gly Leu Glu Thr Gln Trp Leu Lys Asp Glu Leu Glu Ser Gly Pro Asn
      60                  65                  70

TGG AAG CTG TTT GAG CTG AGC GAG ATC GGG GAG GAC AGC AGT CCG CTG        292
Trp Lys Leu Phe Glu Leu Ser Glu Ile Gly Glu Asp Ser Ser Pro Leu
  75                  80                  85                  90
```

```
TGC TTT GAG AAC TGT GGC ACC GTG CAG TCG TCC GCT TCC GCT ACC ATC      340
Cys Phe Glu Asn Cys Gly Thr Val Gln Ser Ser Ala Ser Ala Thr Ile
                 95                  100                 105

ACC GTG TAT TCG TTT CCG GAG AGT GTG GAG CTG AGA CCT CTA CCA GCC      388
Thr Val Tyr Ser Phe Pro Glu Ser Val Glu Leu Arg Pro Leu Pro Ala
            110                 115                 120

TGG CAG CAA GTA GGC AAG GAC CTC ACC CTG CGC TGC CAC GTG GAT GGT      436
Trp Gln Gln Val Gly Lys Asp Leu Thr Leu Arg Cys His Val Asp Gly
        125                 130                 135

GGA GCA CCG CGG ACC CAG CTC TCA GCA GTG CTG CTC CGT GGG GAG GAG      484
Gly Ala Pro Arg Thr Gln Leu Ser Ala Val Leu Leu Arg Gly Glu Glu
    140                 145                 150

ATA CTG AGC CGC CAG CCA GTG GGT GGG CAC CCC AAG GAC CCC AAG GAG      532
Ile Leu Ser Arg Gln Pro Val Gly Gly His Pro Lys Asp Pro Lys Glu
155                 160                 165                 170

ATC ACA TTC ACG GTG CTG GCT AGC AGA GGG GAC CAC GGA GCC AAT TTC      580
Ile Thr Phe Thr Val Leu Ala Ser Arg Gly Asp His Gly Ala Asn Phe
                175                 180                 185

TCA TGC CGC ACA GAA CTG GAT CTC AGG CCG CAA GGG CTG GCA TTG TTC      628
Ser Cys Arg Thr Glu Leu Asp Leu Arg Pro Gln Gly Leu Ala Leu Phe
            190                 195                 200

TCT AAT GTC TCC GAG GCC AGG AGC CTC CGG ACT TTC GAT CTT CCA GCT      676
Ser Asn Val Ser Glu Ala Arg Ser Leu Arg Thr Phe Asp Leu Pro Ala
        205                 210                 215

ACC ATC CCA AAG CTC GAC ACC CCT GAC CTC CTG GAG GTG GGC ACC CAG      724
Thr Ile Pro Lys Leu Asp Thr Pro Asp Leu Leu Glu Val Gly Thr Gln
    220                 225                 230

CAG AAG TTG TTT TGC TCC CTG GAA GGC CTG TTT CCT GCC TCT GAA GCT      772
Gln Lys Leu Phe Cys Ser Leu Glu Gly Leu Phe Pro Ala Ser Glu Ala
235                 240                 245                 250

CGG ATA TAC CTG GAG CTG GGA GGC CAG ATG CCG ACC CAG GAG AGC ACA      820
Arg Ile Tyr Leu Glu Leu Gly Gly Gln Met Pro Thr Gln Glu Ser Thr
                255                 260                 265

AAC AGC AGT GAC TCT GTG TCA GCC ACT GCC TTG GTA GAG GTG ACT GAG      868
Asn Ser Ser Asp Ser Val Ser Ala Thr Ala Leu Val Glu Val Thr Glu
            270                 275                 280

GAG TTC GAC AGA ACC CTG CCG CTG CGC TGC GTT TTG GAG CTA GCG GAC      916
Glu Phe Asp Arg Thr Leu Pro Leu Arg Cys Val Leu Glu Leu Ala Asp
        285                 290                 295

CAG ATC CTG GAG ACG CAG AGG ACC TTA ACA GTC TAC AAC TTT TCA GCT      964
Gln Ile Leu Glu Thr Gln Arg Thr Leu Thr Val Tyr Asn Phe Ser Ala
    300                 305                 310

CCG GTC CTG ACC CTG AGC CAG CTG GAG GTC TCG GAA GGG AGC CAA GTA     1012
Pro Val Leu Thr Leu Ser Gln Leu Glu Val Ser Glu Gly Ser Gln Val
315                 320                 325                 330

ACT GTG AAG TGT GAA GCC CAC AGT GGG TCG AAG GTG GTT CTT CTG AGC     1060
Thr Val Lys Cys Glu Ala His Ser Gly Ser Lys Val Val Leu Leu Ser
                335                 340                 345

GGC GTC GAG CCT AGG CCA CCC ACC CCG CAG GTC CAA TTC ACA CTG AAT     1108
Gly Val Glu Pro Arg Pro Pro Thr Pro Gln Val Gln Phe Thr Leu Asn
            350                 355                 360
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```
GCC AGC TCG GAG GAT CAC AAA CGA AGC TTC TTT TGC TCT GCC GCT CTG      1156
Ala Ser Ser Glu Asp His Lys Arg Ser Phe Phe Cys Ser Ala Ala Leu
        365                 370                 375

GAG GTG GCG GGA AAG TTC CTG TTT AAA AAC CAG ACC CTG GAA CTG CAC      1204
Glu Val Ala Gly Lys Phe Leu Phe Lys Asn Gln Thr Leu Glu Leu His
    380                 385                 390

GTG CTG TAT GGT CCT CGG CTG GAC GAG ACG GAC TGC TTG GGG AAC TGG      1252
Val Leu Tyr Gly Pro Arg Leu Asp Glu Thr Asp Cys Leu Gly Asn Trp
395                 400                 405                 410

ACC TGG CAA GAG GGG TCT CAG CAG ACT CTG AAA TGC CAG GCC TGG GGG      1300
Thr Trp Gln Glu Gly Ser Gln Gln Thr Leu Lys Cys Gln Ala Trp Gly
                415                 420                 425

AAC CCA TCT CCT AAA ATG ACC TGC AGA CGG AAG GCA GAT GGT GCC CTG      1348
Asn Pro Ser Pro Lys Met Thr Cys Arg Arg Lys Ala Asp Gly Ala Leu
            430                 435                 440

CTG CCC ATC GGG GTG GTG AAG TCT GTC AAA CAG GAG ATG AAT GGT ACA      1396
Leu Pro Ile Gly Val Val Lys Ser Val Lys Gln Glu Met Asn Gly Thr
        445                 450                 455

TAC GTG TGC CAT GCC TTT AGC TCC CAT GGG AAT GTC ACC AGG AAT GTG      1444
Tyr Val Cys His Ala Phe Ser Ser His Gly Asn Val Thr Arg Asn Val
    460                 465                 470

TAC CTG ACA GTA CTG TAC CAC TCT CAA AAT AAC TGG ACT ATA ATC ATT      1492
Tyr Leu Thr Val Leu Tyr His Ser Gln Asn Asn Trp Thr Ile Ile Ile
475                 480                 485                 490

CTG GTG CCA GTA CTG CTG GTC ATT GTG GGC CTC GTG ATG GCA GCC TCT      1540
Leu Val Pro Val Leu Leu Val Ile Val Gly Leu Val Met Ala Ala Ser
                495                 500                 505

TAT GTT TAT AAC CGC CAG AGA AAG ATC AGG ATA TAC AAG TTA CAG AAG      1588
Tyr Val Tyr Asn Arg Gln Arg Lys Ile Arg Ile Tyr Lys Leu Gln Lys
            510                 515                 520

GCT CAG GAG GAG GCC ATA AAA CTC AAG GGA CAA GCC CCA CCT CCC          1633
Ala Gln Glu Glu Ala Ile Lys Leu Lys Gly Gln Ala Pro Pro Pro
        525                 530                 535

TGAGCCTGCT GGATGAGACT CCTGCCTGGA CCCCCTGCAG GGCAACAGCT GCTGCTGCTT    1693

TTGAACAGAA TGGTAGACAG CATTTACCCT CAGCCACTTC CTCTGGCTGT CACAGAACAG    1753

GATGGTGGCC TGGGGGATGC ACACTTGTAG CCTCAGAGCT AAGAGGACTC GGTGGATGGA    1813

GCAAGACTGT GAACACGTGT GACCCGGACC CACCTACAGC CCGGTGGACC TTCAGCCAAG    1873

AAACGCTGAC TTCATTCTCT ATTGCCCCTG CTGAGGGGCT CCTGCCTAAG GAAGACATGA    1933

TATCCAGTAG ACACAAGCAA GAAGACCACA CTTCCCCCCC GACACAGGAA AGCTGAGACA    1993

TTGTCCCCAA CTCTTCTTGA TGTATTTATT AATTTAGAGT TTTACCAGCT ATTTATTGAG    2053

TACCCTGTAT ATAGTAGATC AGTGAGGAGG TGAATGTATA AGTTATGGCC TGGACCCTGC    2113

TGCAGATGCT GTGAGAGTCT GGGGAAAGAT CACATGGGTC GAGGGTTTCT CTACTGGTCA    2173

GGATGCTTTT CTCATAAGGG TCGACTTTTT TCACCAGTCA CATAAACACT ATGTGGACTA    2233

GCAGTGGTTC TCTGCTCCTC CACATCCTGG AGCGTCCCAG CACCTCCCCA CCTACTTTTG    2293

TTCCCAATGT CAGCCACCAT GCCTTAGCAG CTGAACAATC GAGCCTCATG CTCATGAAAT    2353
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CATGGTCCCA GGCGGCTCCA CCTCAAAGAG AAAGCCTGGA AGGAAATGTT CCAACTCCTT 2413

AGAAGGGTCG TGCAAGCTGC TGTGGGAGGG TAAGCACCCC TCCCAGCAGC AGAAACCTTT 2473

CCTTTGAATC AATAAAGTTT TATGTCGGCC TGAAAAAAAA AAAAAAAAA 2522

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 537 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Met Ala Ser Thr Arg Ala Lys Pro Thr Leu Pro Leu Leu Leu Ala Leu
1 5 10 15

Val Thr Val Val Ile Pro Gly Pro Gly Asp Ala Gln Val Ser Ile His
20 25 30

Pro Arg Glu Ala Phe Leu Pro Gln Gly Gly Ser Val Gln Val Asn Cys
35 40 45

Ser Ser Ser Cys Lys Glu Asp Leu Ser Leu Gly Leu Glu Thr Gln Trp
50 55 60

Leu Lys Asp Glu Leu Glu Ser Gly Pro Asn Trp Lys Leu Phe Glu Leu
65 70 75 80

Ser Glu Ile Gly Glu Asp Ser Ser Pro Leu Cys Phe Glu Asn Cys Gly
85 90 95

Thr Val Gln Ser Ser Ala Ser Ala Thr Ile Thr Val Tyr Ser Phe Pro
100 105 110

Glu Ser Val Glu Leu Arg Pro Leu Pro Ala Trp Gln Gln Val Gly Lys
115 120 125

Asp Leu Thr Leu Arg Cys His Val Asp Gly Gly Ala Pro Arg Thr Gln
130 135 140

Leu Ser Ala Val Leu Leu Arg Gly Glu Glu Ile Leu Ser Arg Gln Pro
145 150 155 160

Val Gly Gly His Pro Lys Asp Pro Lys Glu Ile Thr Phe Thr Val Leu
165 170 175

Ala Ser Arg Gly Asp His Gly Ala Asn Phe Ser Cys Arg Thr Glu Leu
180 185 190

Asp Leu Arg Pro Gln Gly Leu Ala Leu Phe Ser Asn Val Ser Glu Ala
195 200 205

Arg Ser Leu Arg Thr Phe Asp Leu Pro Ala Thr Ile Pro Lys Leu Asp
210 215 220

Thr Pro Asp Leu Leu Glu Val Gly Thr Gln Gln Lys Leu Phe Cys Ser
225 230 235 240

Leu Glu Gly Leu Phe Pro Ala Ser Glu Ala Arg Ile Tyr Leu Glu Leu
245 250 255

```
Gly Gly Gln Met Pro Thr Gln Glu Ser Thr Asn Ser Ser Asp Ser Val
            260                 265                 270

Ser Ala Thr Ala Leu Val Glu Val Thr Glu Glu Phe Asp Arg Thr Leu
        275                 280                 285

Pro Leu Arg Cys Val Leu Glu Leu Ala Asp Gln Ile Leu Glu Thr Gln
    290                 295                 300

Arg Thr Leu Thr Val Tyr Asn Phe Ser Ala Pro Val Leu Thr Leu Ser
305                 310                 315                 320

Gln Leu Glu Val Ser Glu Gly Ser Gln Val Thr Val Lys Cys Glu Ala
                325                 330                 335

His Ser Gly Ser Lys Val Val Leu Leu Ser Gly Val Glu Pro Arg Pro
            340                 345                 350

Pro Thr Pro Gln Val Gln Phe Thr Leu Asn Ala Ser Ser Glu Asp His
        355                 360                 365

Lys Arg Ser Phe Phe Cys Ser Ala Ala Leu Glu Val Ala Gly Lys Phe
    370                 375                 380

Leu Phe Lys Asn Gln Thr Leu Glu Leu His Val Leu Tyr Gly Pro Arg
385                 390                 395                 400

Leu Asp Glu Thr Asp Cys Leu Gly Asn Trp Thr Trp Gln Glu Gly Ser
                405                 410                 415

Gln Gln Thr Leu Lys Cys Gln Ala Trp Gly Asn Pro Ser Pro Lys Met
            420                 425                 430

Thr Cys Arg Arg Lys Ala Asp Gly Ala Leu Leu Pro Ile Gly Val Val
        435                 440                 445

Lys Ser Val Lys Gln Glu Met Asn Gly Thr Tyr Val Cys His Ala Phe
    450                 455                 460

Ser Ser His Gly Asn Val Thr Arg Asn Val Tyr Leu Thr Val Leu Tyr
465                 470                 475                 480

His Ser Gln Asn Asn Trp Thr Ile Ile Ile Leu Val Pro Val Leu Leu
                485                 490                 495

Val Ile Val Gly Leu Val Met Ala Ala Ser Tyr Val Tyr Asn Arg Gln
            500                 505                 510

Arg Lys Ile Arg Ile Tyr Lys Leu Gln Lys Ala Gln Glu Glu Ala Ile
        515                 520                 525

Lys Leu Lys Gly Gln Ala Pro Pro Pro
    530                 535
```

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GCCCAAGCTG GCATCCGTCA 20

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

TGCATCCCCC AGGCCACCAT 20

WHAT IS CLAIMED IS:

1. A pharmaceutical composition for the treatment of pathologic conditions associated with the overexpression of ICAM-1 in a host, said pharmaceutical composition comprising an effective amount of an ICAM-1 antisense molecule encapsulated in a lipid mixture, said lipid mixture comprising at least two members selected from the group consisting of phospholipids, sterols and cationic lipids.

2. A pharmaceutical composition in accordance with claim 1, wherein said lipid mixture is in the form of liposomes and consists essentially of neutral phospholipids and a sterol.

3. A pharmaceutical composition in accordance with claim 1, wherein said lipid mixture is in the form of lipid particles.

4. A pharmaceutical composition in accordance with claim 1, wherein said ICAM-1 antisense molecule comprises from about 15 to about 50 nucleic acids and is complementary to a portion of the 3'-untranslated region of ICAM-1.

5. A pharmaceutical composition in accordance with claim 4, wherein said ICAM-1 antisense molecule is a phosphorothioate molecule.

6. A pharmaceutical composition in accordance with claim 4, wherein said ICAM-1 antisense molecule is a methyl phosphonate molecule.

7. A pharmaceutical composition in accordance with claim 1, wherein said lipid mixture is in the form of liposomes consisting essentially of phosphatidylcholine and a sterol.

8. A pharmaceutical composition in accordance with claim 1, wherein said lipid mixture is in the form of liposomes consisting essentially of egg phosphatidylcholine and cholesterol.

9. A pharmaceutical composition in accordance with claim 1, wherein said lipid mixture is in the form of liposomes consisting essentially of neutral phospholipids and cholesterol, said liposomes having diameters of from about 50 to about 150 nm.

10. A pharmaceutical composition in accordance with claim 9, wherein said liposomes have diameters of from about 75 to about 125 nm.

11. A pharmaceutical composition in accordance with claim 1, wherein said lipid mixture is in the form of lipid particles, said lipid particles being from about 50 to about 90 nm in diameter.

12. A pharmaceutical composition in accordance with claim 1, wherein said lipid mixture is in the form of lipid particles, said lipid particles being from about 60 to about 80 nm in diameter.

13. A pharmaceutical composition in accordance with claim 12, wherein said lipid particles comprise phospholipids and cationic lipids, wherein said cationic lipids are members selected from the group consisting of DODAC, DDAB, DOTAP, DOTMA, DOSPA, DOGS, DC-Chol and combinations thereof, and said phospholipids are members selected from the group consisting of DOPE, POPC, EPC and combinations thereof.

14. A method for the treatment of pathologic conditions associated with the overexpression of ICAM-1 in a host, said method comprising delivering to said host a pharmaceutical composition in accordance with claim 1.

15. A method in accordance with claim 14, wherein said composition is a liposomal composition.

16. A method in accordance with claim 14, wherein said composition comprises lipid particles.

17. A method in accordance with claim 14, wherein said ICAM-1 antisense molecule comprises from about 15 to about 50 nucleic acids and is complementary to a portion of the 3'-untranslated region of ICAM-1.

18. A method in accordance with claim 14, wherein said delivering comprises administering intravenously.

19. A method in accordance with claim 14, wherein said delivering comprises administering topically.

20. A method in accordance with claim 16, wherein said lipid particles comprise phospholipids and cationic lipids, said cationic lipids being members selected from the group consisting of DODAC, DDAB, DOTAP, DOTMA, DOSPA, DOGS, DC-Chol and combinations thereof, and said phospholipids being members selected from the group consisting of DOPE, POPC, EPC and combinations thereof.

21. A method in accordance with claim 14, wherein said condition is a member selected from the group consisting of Alzheimer's disease, multiple sclerosis, uveitis, Herpes keratitis, renal allograft rejection, glomerulonephritis, liver allograft rejection, viral hepatitis, alcoholic hepatitis, cholangitis, cardiac allograft rejection, atherosclerotic plaques, rheumatoid arthritis, Grave's disease, Hashimoto's thyroiditis, psoriasis, scleroderma, graft v host disease, contact dermatitis, lichen planus, fixed drug eruption, mycosis fungoides, and alopecia areata.

22. A method in accordance with claim 14, wherein said condition is contact dermatitis.

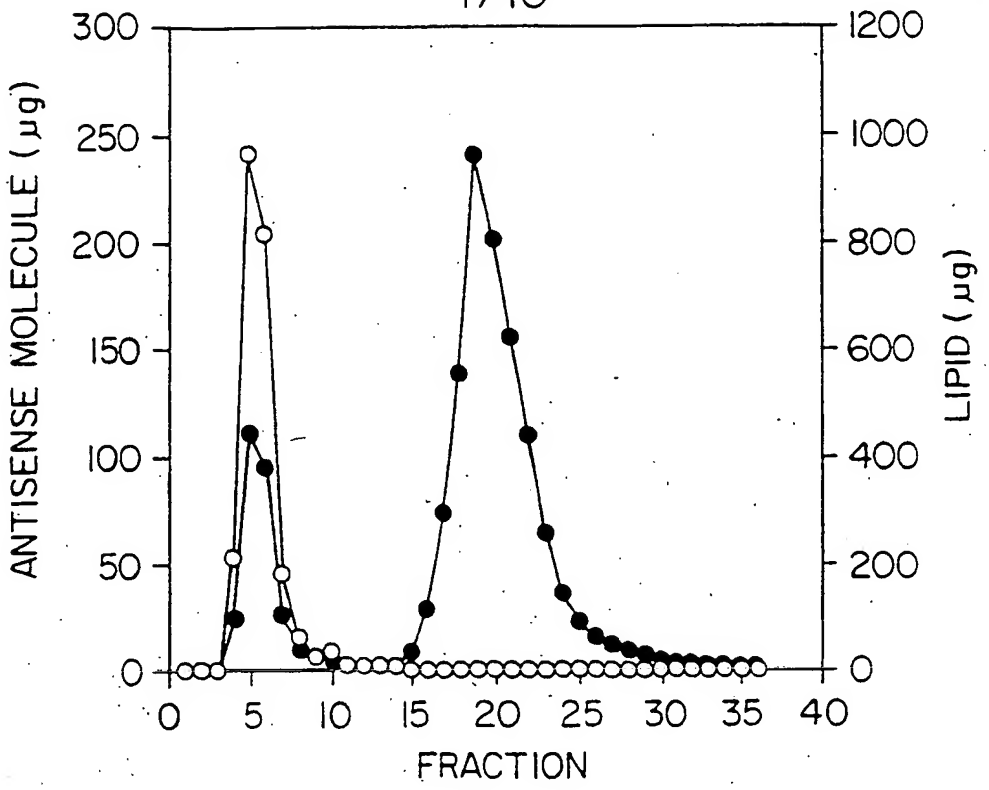
ANTISENSE MOLECULE (µg)
LIPID (µg)
FRACTION
300
250
200
150
100
50
0
1200
1000
800
600
400
200
0
0 5 10 15 20 25 30 35 40

FIG. 1

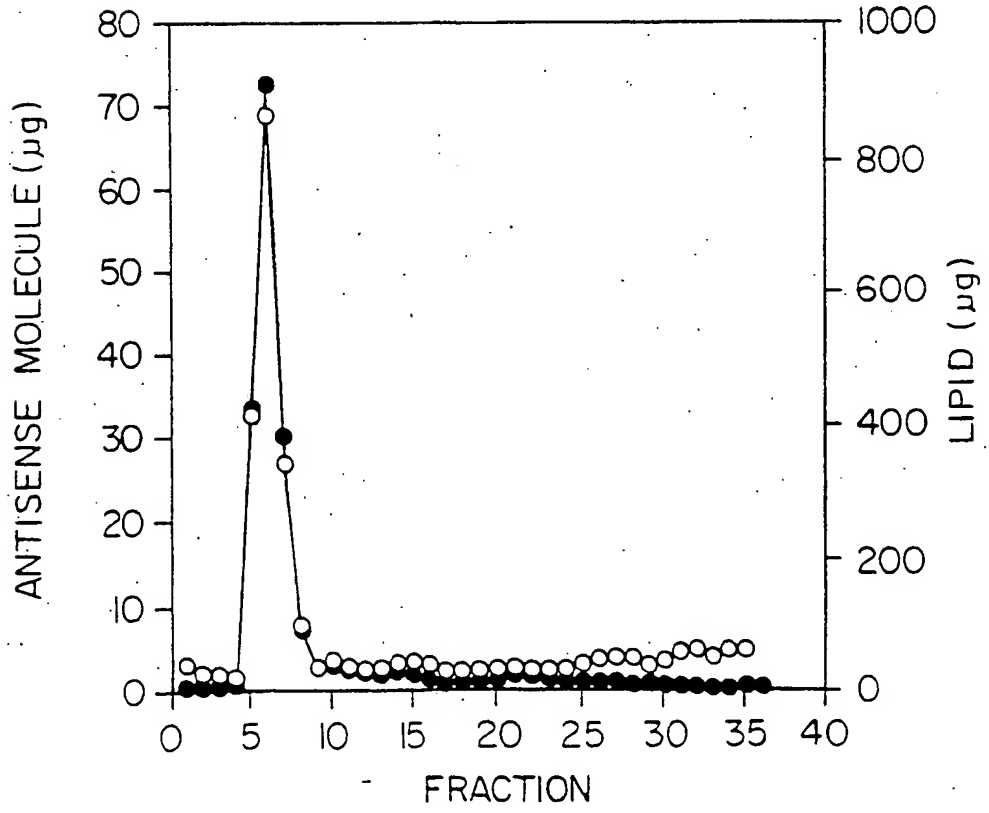
ANTISENSE MOLECULE (µg)
LIPID (µg)
FRACTION
80
70
60
50
40
30
20
10
0
1000
800
600
400
200
0
0 5 10 15 20 25 30 35 40

FIG. 2

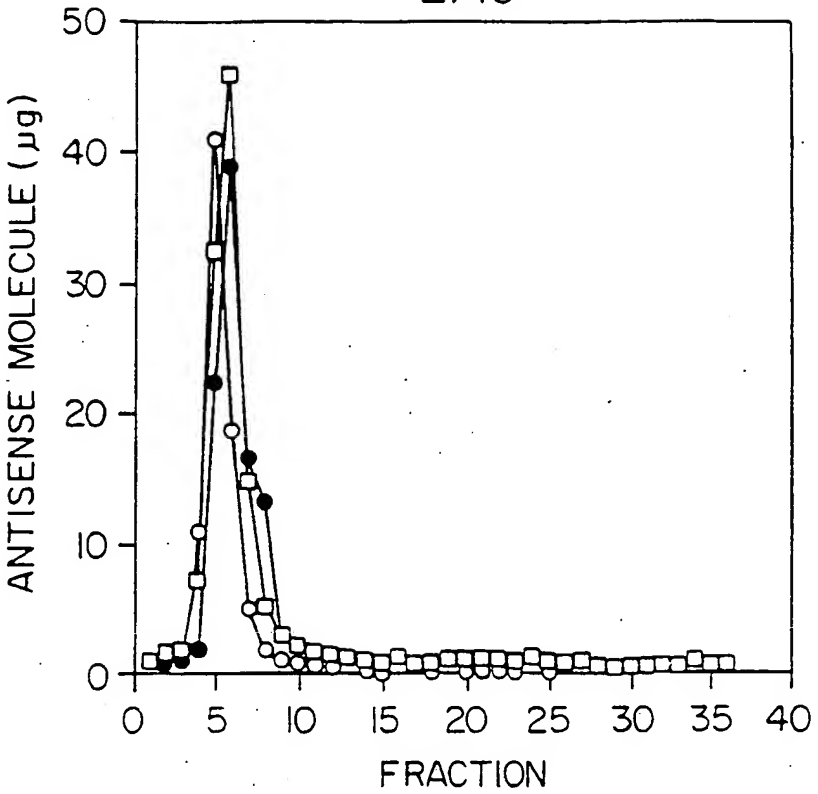
ANTISENSE MOLECULE (µg)
50
40
30
20
10
0
0 5 10 15 20 25 30 35 40
FRACTION

FIG. 3

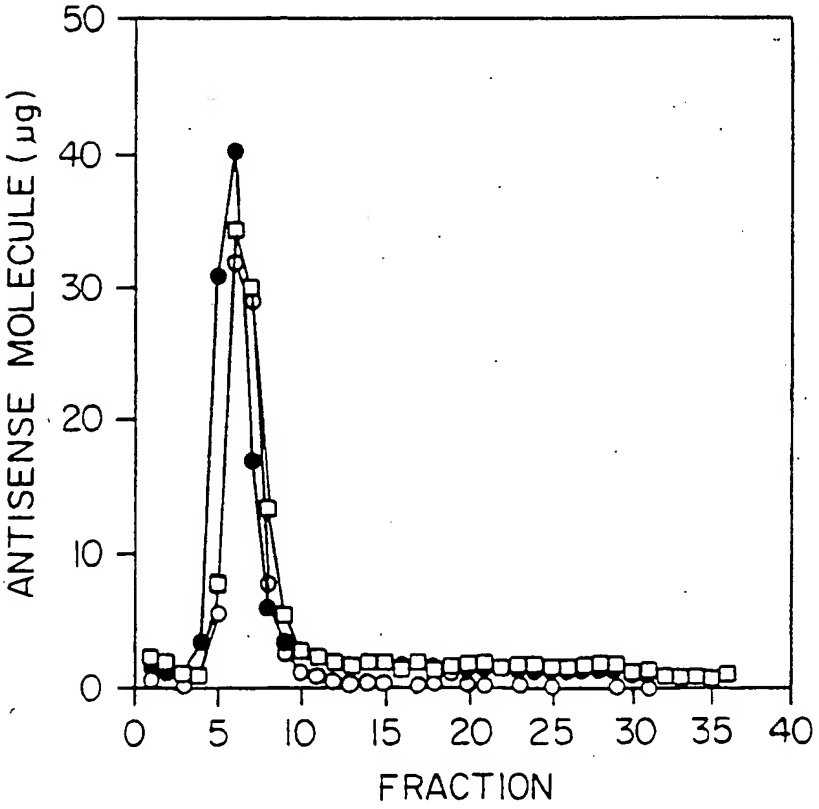
ANTISENSE MOLECULE (µg)
50
40
30
20
10
0
0 5 10 15 20 25 30 35 40
FRACTION

FIG. 4

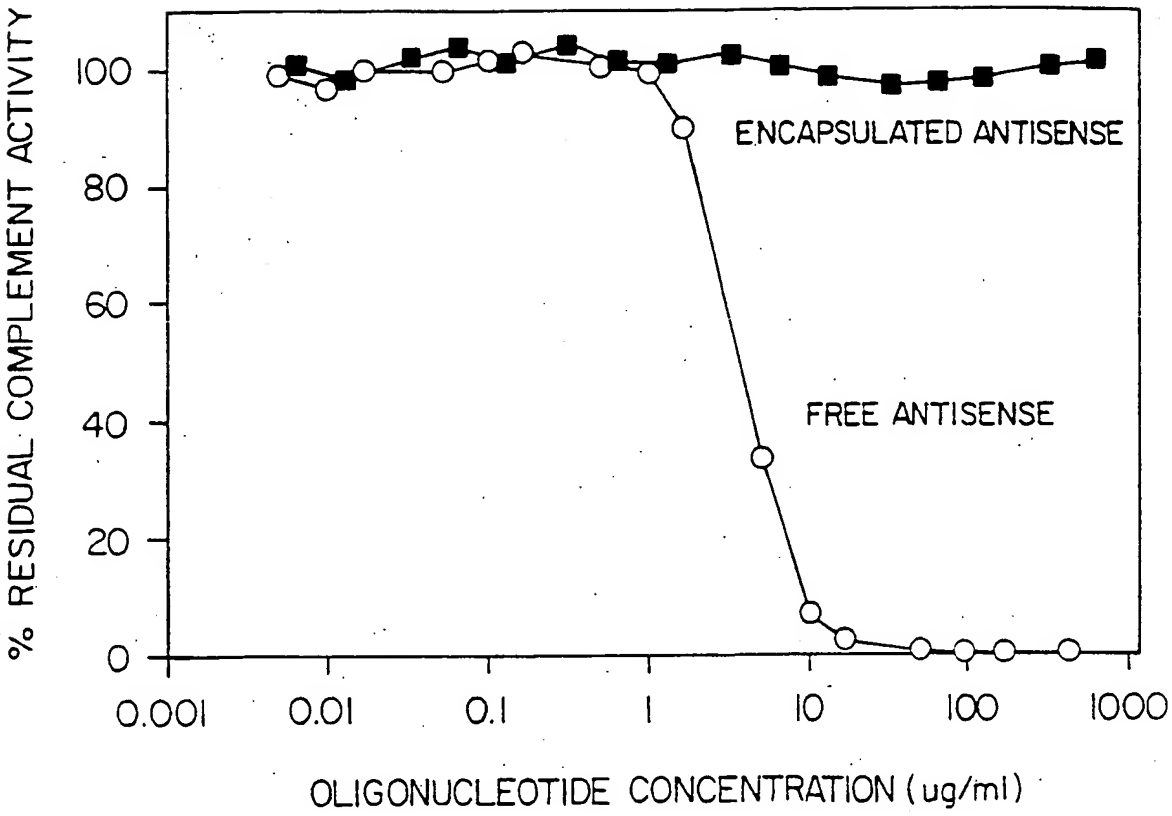
% RESIDUAL COMPLEMENT ACTIVITY
100
80
60
40
20
0
0.001
0.01
0.1
1
10
100
1000
ENCAPSULATED ANTISENSE
FREE ANTISENSE
OLIGONUCLEOTIDE CONCENTRATION (ug/ml)

FIG. 5

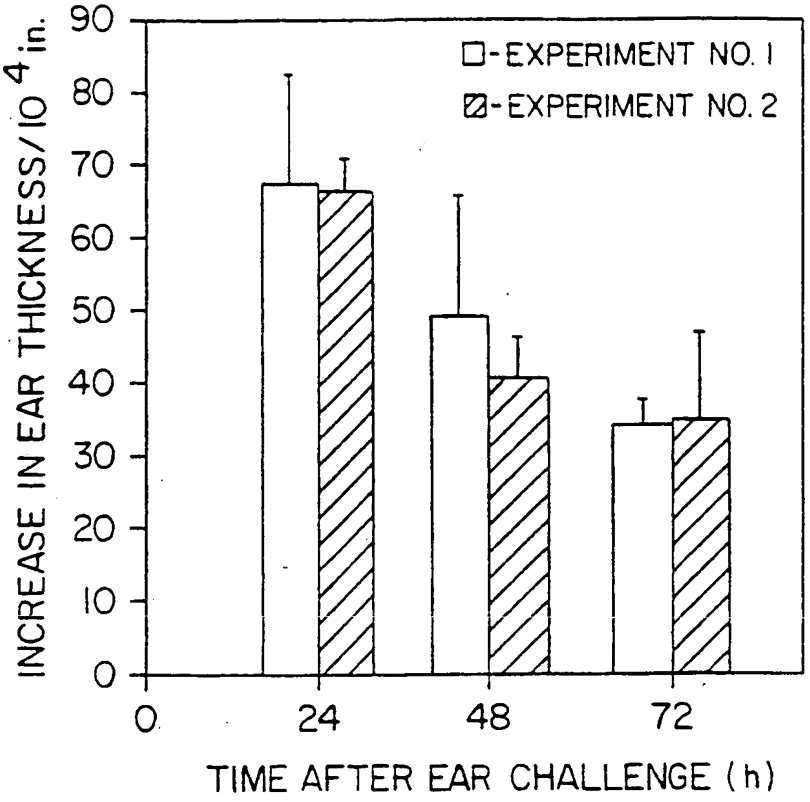
□-EXPERIMENT NO. 1
▨-EXPERIMENT NO. 2
INCREASE IN EAR THICKNESS/10 4 in.
90
80
70
60
50
40
30
20
10
0
0
24
48
72
TIME AFTER EAR CHALLENGE (h)

FIG. 6

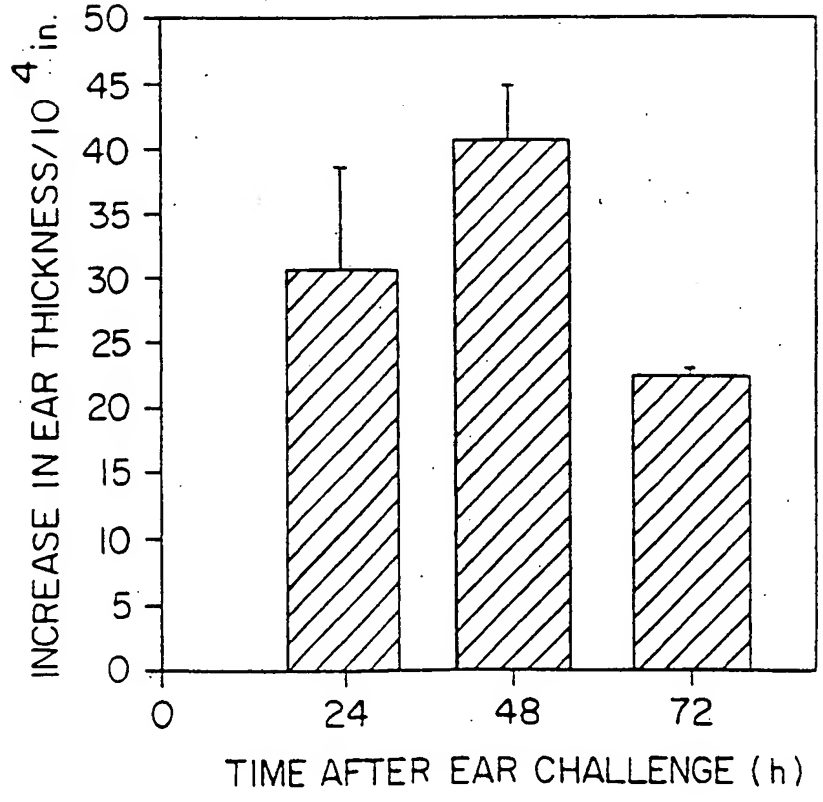
INCREASE IN EAR THICKNESS/10 4 in.
50
45
40
35
30
25
20
15
10
5
0
0
24
48
72
TIME AFTER EAR CHALLENGE (h)

FIG. 7

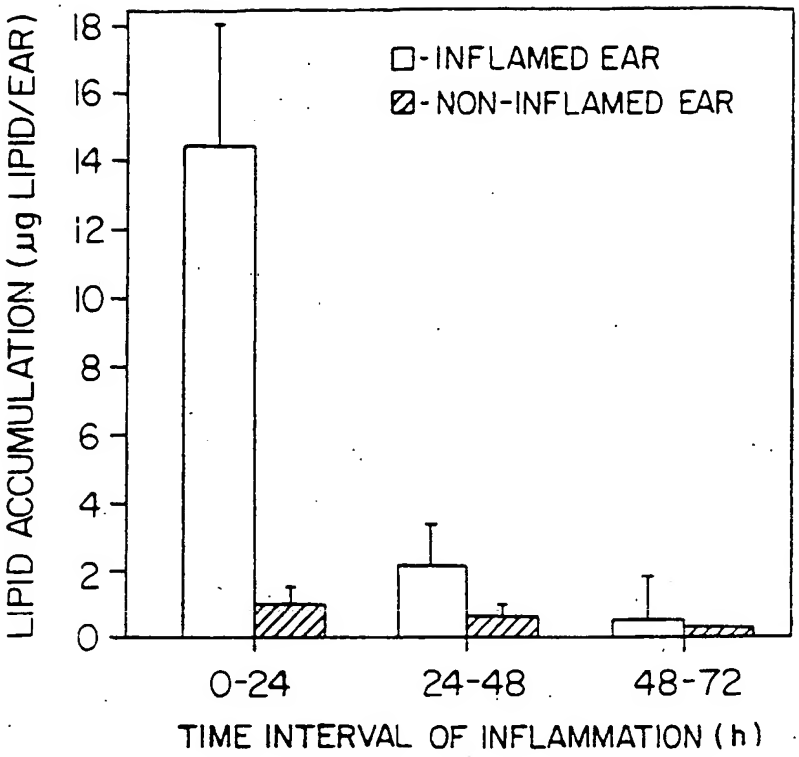
LIPID ACCUMULATION (µg LIPID/EAR)
18
16
14
12
10
8
6
4
2
0
□-INFLAMED EAR
▨-NON-INFLAMED EAR
0-24
24-48
48-72
TIME INTERVAL OF INFLAMMATION (h)

FIG. 8

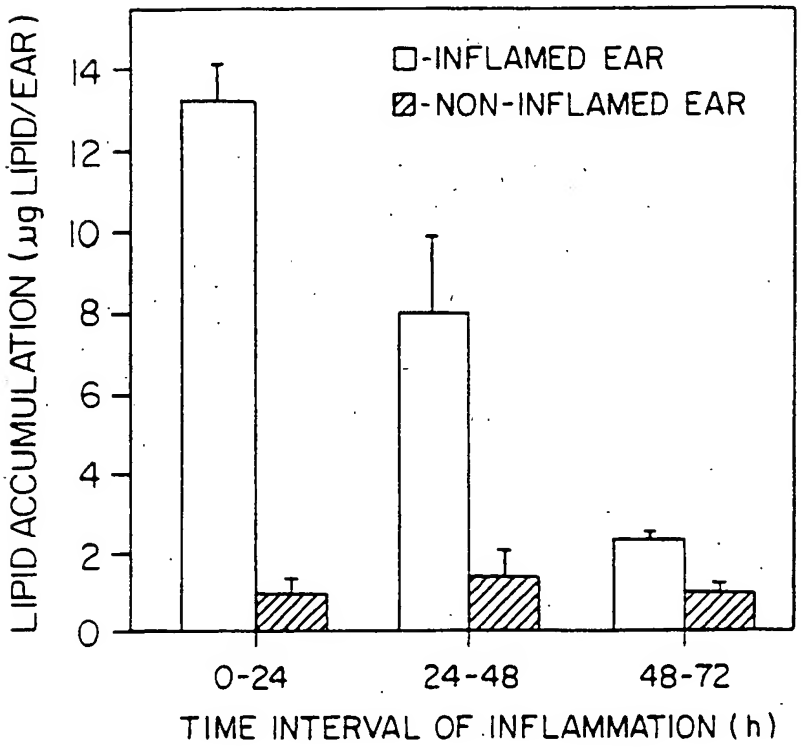
LIPID ACCUMULATION (µg LIPID/EAR)
14
12
10
8
6
4
2
0
□-INFLAMED EAR
▨-NON-INFLAMED EAR
0-24
24-48
48-72
TIME INTERVAL OF INFLAMMATION (h)

FIG. 9

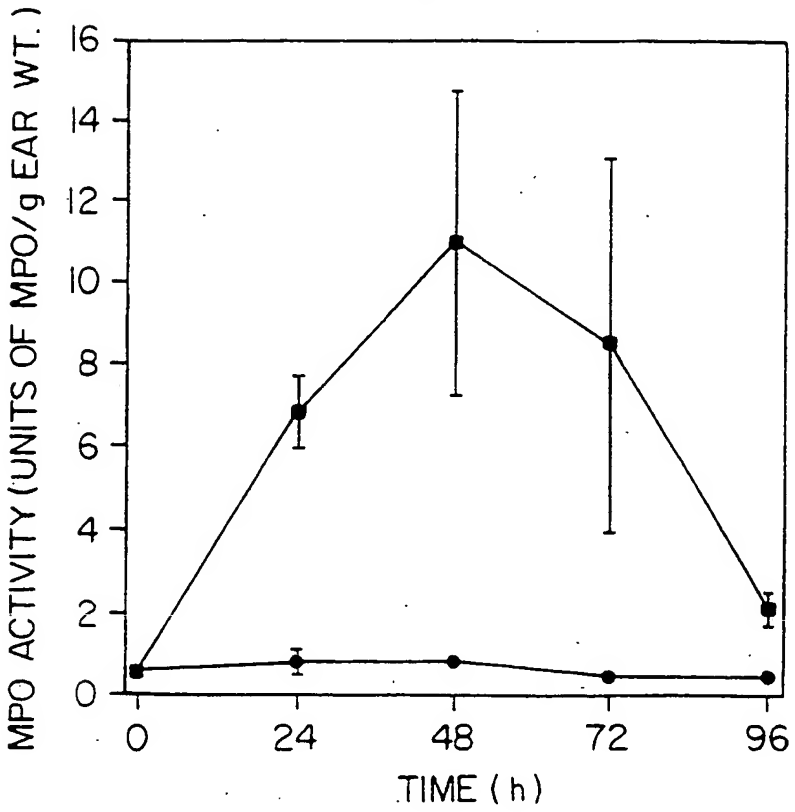
MPO ACTIVITY (UNITS OF MPO/g EAR WT.)
16
14
12
10
8
6
4
2
0
0
24
48
72
96
TIME (h)

FIG. 10

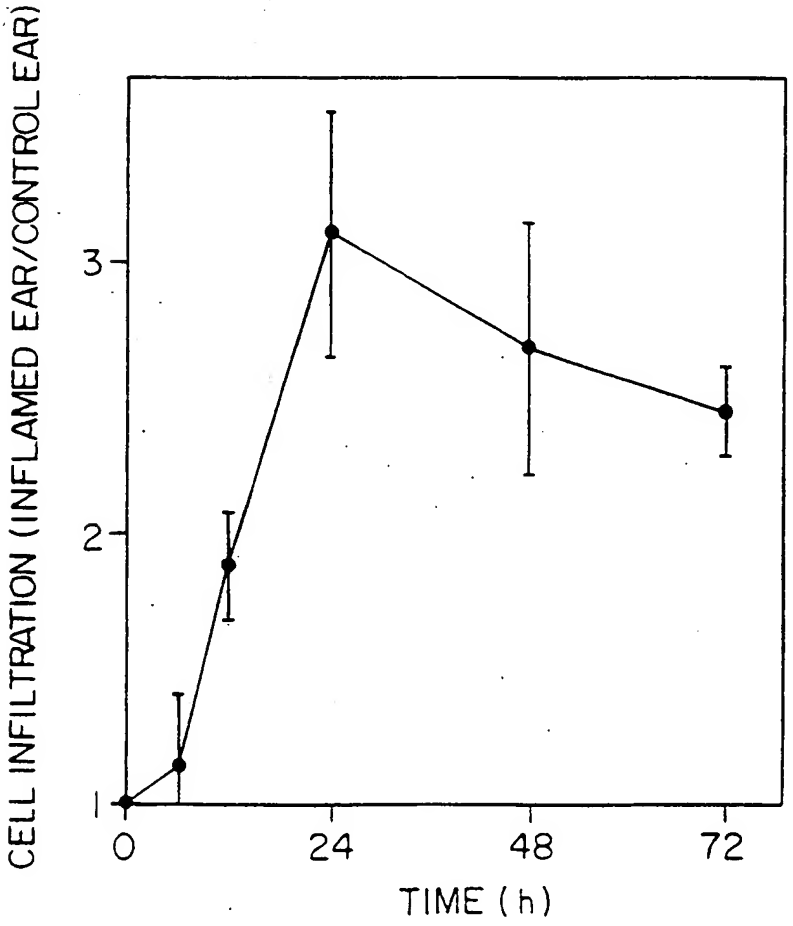
CELL INFILTRATION (INFLAMED EAR/CONTROL EAR)
3
2
1
0
24
48
72
TIME (h)

FIG. 11

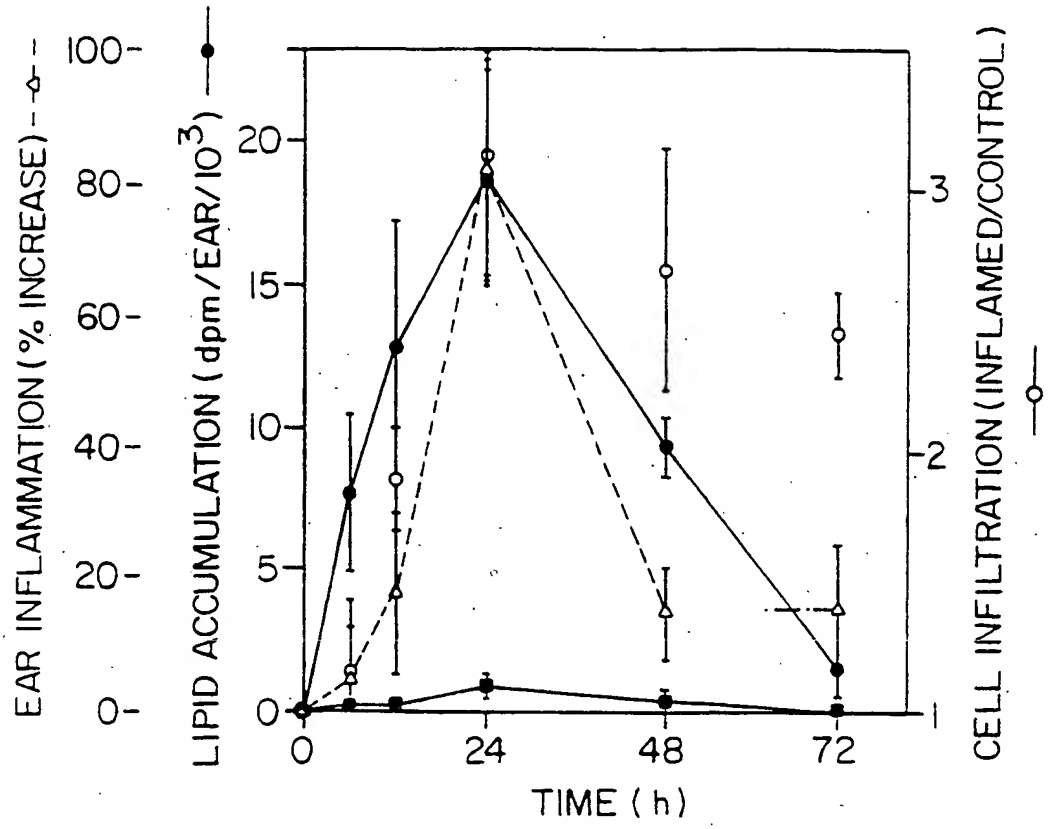
EAR INFLAMMATION (% INCREASE)
LIPID ACCUMULATION (dpm/EAR/10^3)
CELL INFILTRATION (INFLAMED/CONTROL)
100
80
60
40
20
0
20
15
10
5
0
3
2
1
0
24
48
72
TIME (h)

FIG. 12

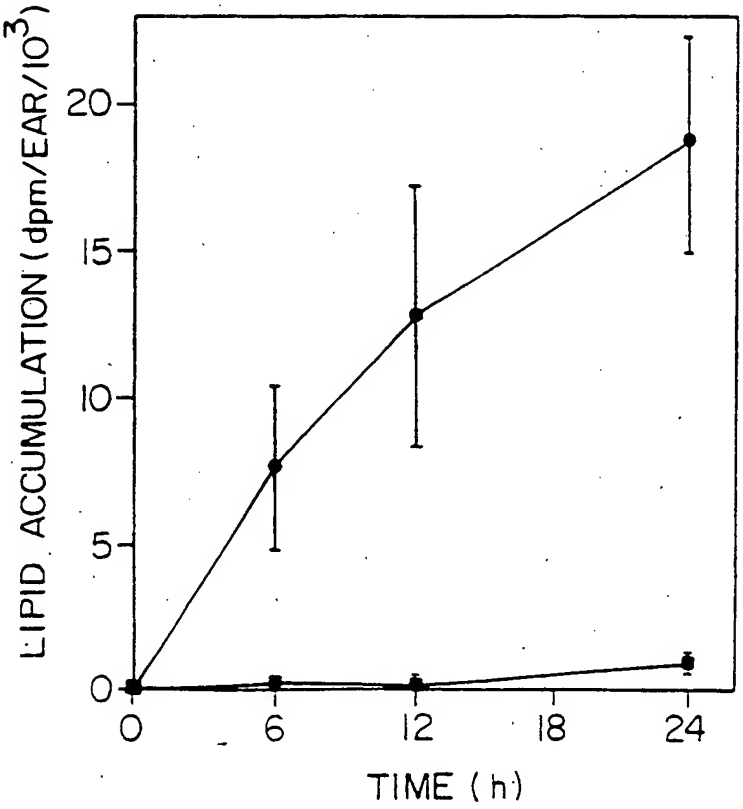
LIPID ACCUMULATION (dpm/EAR/10^3)
20
15
10
5
0
0
6
12
18
24
TIME (h)

FIG. 13

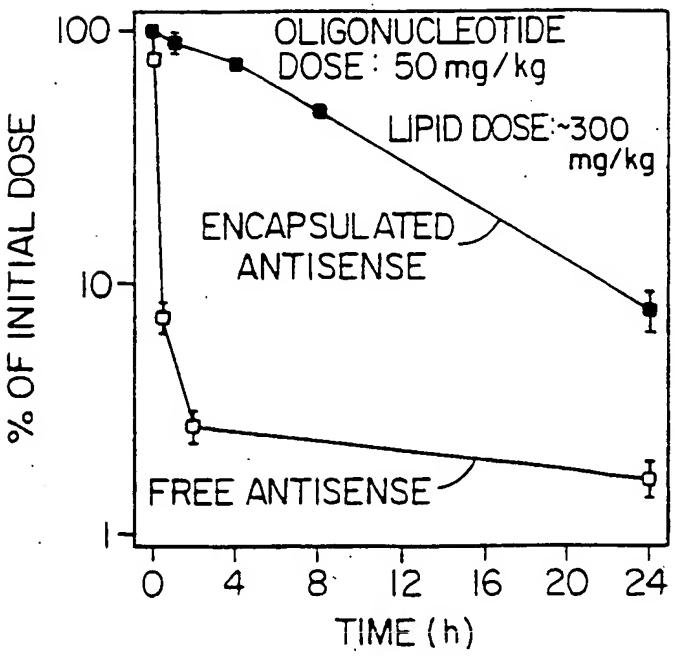
OLIGONUCLEOTIDE
DOSE: 50 mg/kg
LIPID DOSE: ~300 mg/kg
ENCAPSULATED ANTISENSE
FREE ANTISENSE
% OF INITIAL DOSE
100
10
1
0
4
8
12
16
20
24
TIME (h)

FIG. 14

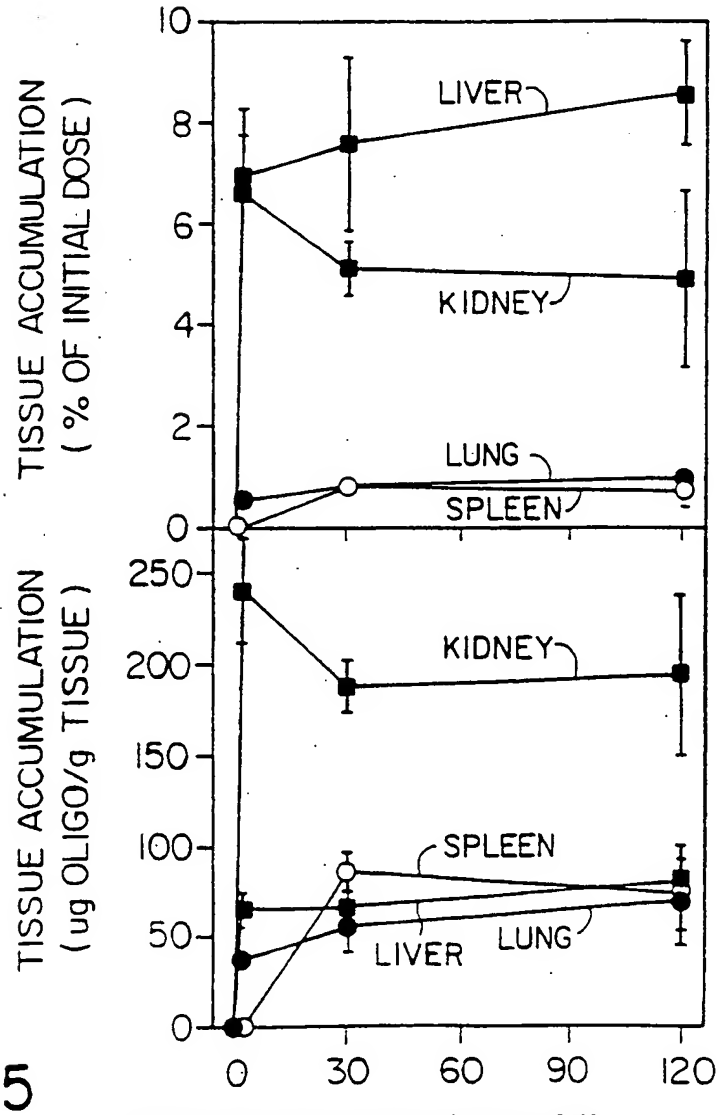
TISSUE ACCUMULATION
(% OF INITIAL DOSE)
LIVER
KIDNEY
LUNG
SPLEEN
10
8
6
4
2
0
TISSUE ACCUMULATION
(ug OLIGO/g TISSUE)
250
200
150
100
50
0
KIDNEY
SPLEEN
LIVER
LUNG
0
30
60
90
120

FIG. 15

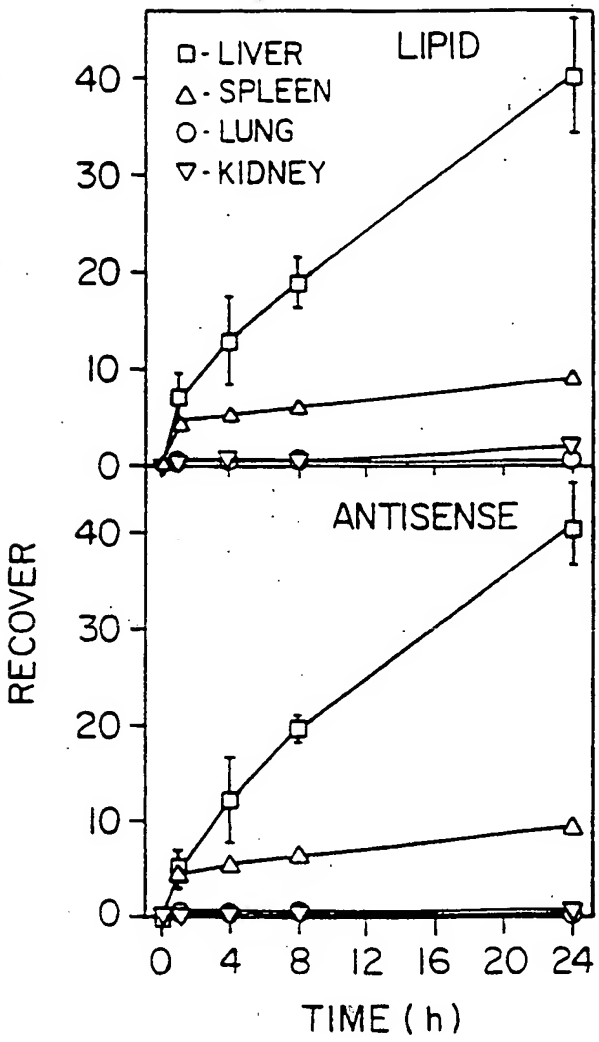
□- LIVER
△- SPLEEN
○- LUNG
▽- KIDNEY
LIPID
ANTISENSE
RECOVER
0
10
20
30
40
0 4 8 12 16 20 24
TIME (h)

FIG. 16

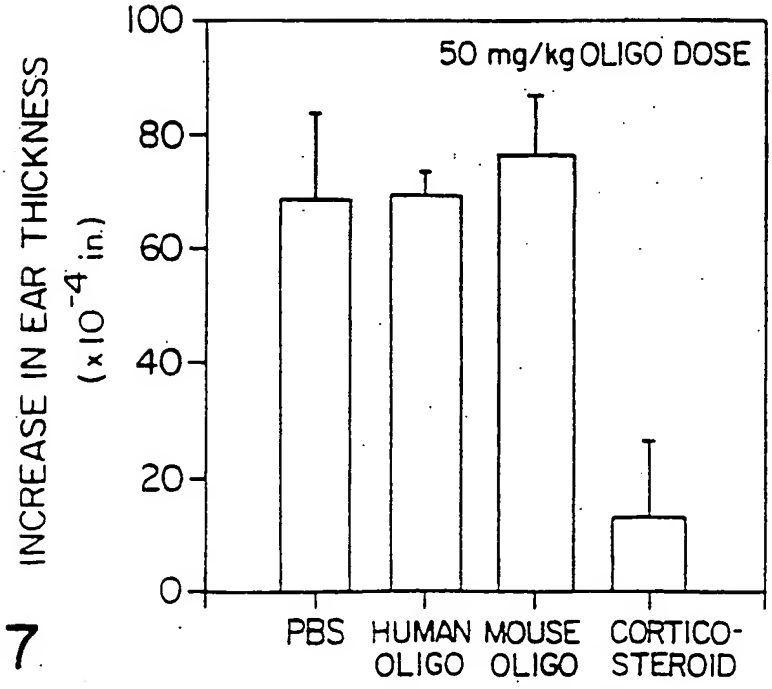
50 mg/kg OLIGO DOSE
INCREASE IN EAR THICKNESS
(x10⁻⁴ in.)
0
20
40
60
80
100
PBS
HUMAN OLIGO
MOUSE OLIGO
CORTICO-STEROID

FIG. 17

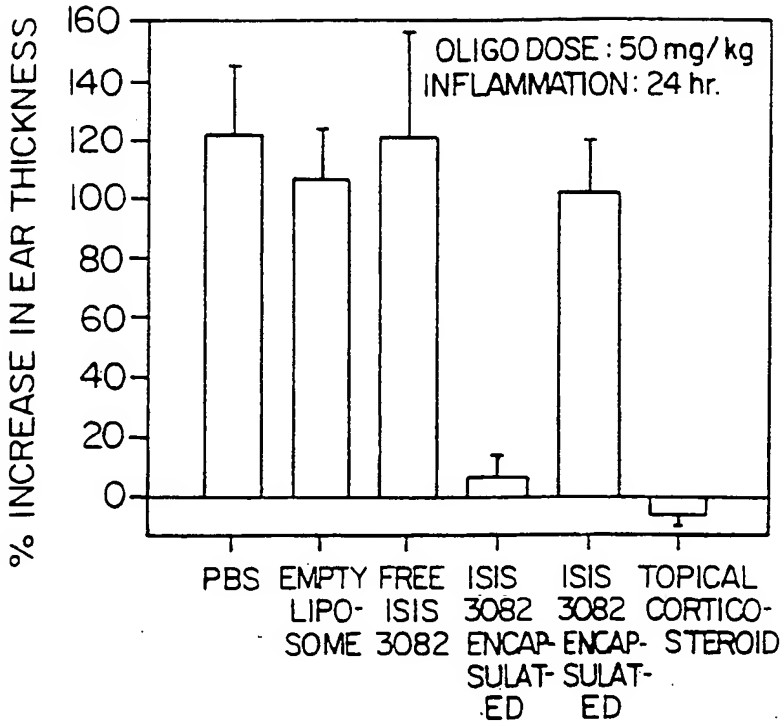
% INCREASE IN EAR THICKNESS
160
140
120
100
80
60
40
20
0
OLIGO DOSE : 50 mg/kg
INFLAMMATION : 24 hr.
PBS
EMPTY LIPO-SOME
FREE ISIS 3082
ISIS 3082 ENCAP-SULAT-ED
ISIS 3082 ENCAP-SULAT-ED
TOPICAL CORTICO-STEROID

FIG. 18

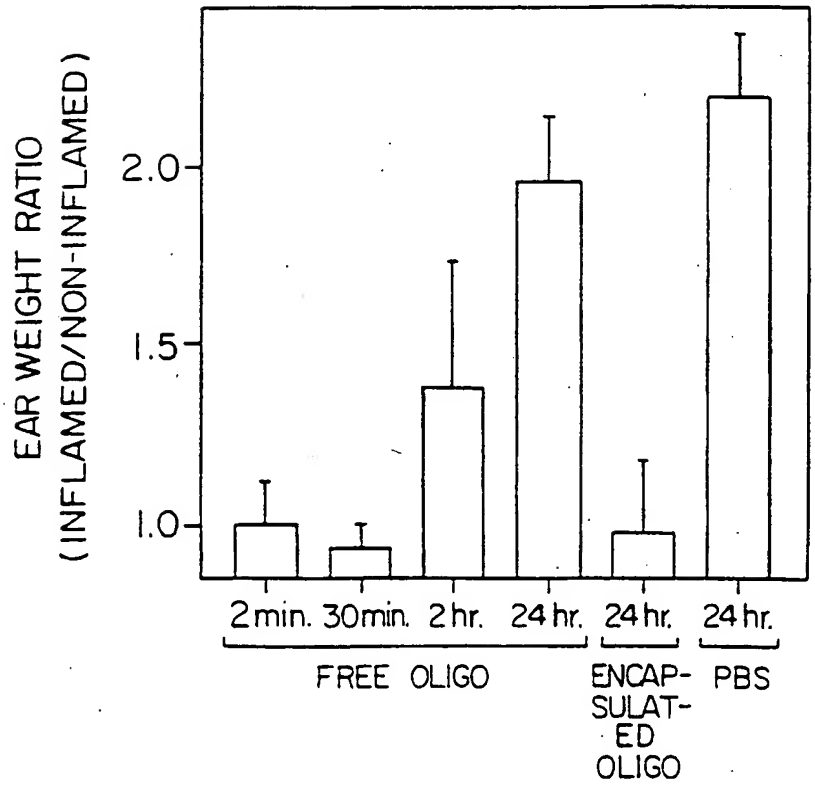
EAR WEIGHT RATIO
(INFLAMED/NON-INFLAMED)
2.0
1.5
1.0
2 min.
30 min.
2 hr.
24 hr.
24 hr.
24 hr.
FREE OLIGO
ENCAP-SULAT-ED OLIGO
PBS

FIG. 19

INTERNATIONAL SEARCH REPORT

Inten ial Application No
PCT/CA 97/00347

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/11 A61K9/127 A61K31/70 C07H21/00 //C12N15/88

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N A61K C07H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 94 05333 A (ISIS PHARMACEUTICALS INC) 17 March 1994 cited in the application see page 4, line 10 - page 5, line 8 see page 11, line 28 - page 14, line 17 see page 26 - page 30, line 4	1,3-6, 14,16-22
Y	see examples 15-22	1-5, 7-17,19, 20

Y	WO 96 10390 A (INEX PHARMACEUTICALS CORP) 11 April 1996 cited in the application see page 9, line 14 - line 30 see page 12, line 20 - line 28 see examples 3-9	1,3-5, 11-14, 16,17,20

	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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1

Date of the actual completion of the international search	Date of mailing of the international search report
10 September 1997	30.09.97

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Andres, S

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	JOURNAL OF CONTROLLED RELEASE, vol. 41, no. 1/2, August 1996, pages 121-130, XP000592989 BENNETT, C. ET AL.: "PHARMACOKINETICS IN MICE OF A 3H-LABELED PHOSPHOROTHIOATE OLIGONUCLEOTIDE FORMULATED IN THE PRESENCE AND ABSENCE OF A CATIONIC LIPID" see the whole document	1,3-5, 14, 16-18, 20,21

INTERNATIONAL SEARCH REPORT

...formation on patent family members

Inter ...al Application No
PCT/CA 97/00347

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		EP 0662003 A	12-07-95
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		JP 8500736 T	30-01-96
		NO 950800 A	24-04-95
		NZ 256171 A	20-12-96
WO 9610390 A	11-04-96	AU 3559595 A	26-04-96
		EP 0783296 A	16-07-97
WO 9422468 A	13-10-94	US 5641508 A	24-06-97
		AU 6554594 A	24-10-94
		CA 2159626 A	13-10-94
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		JP 8511510 T	03-12-96